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Digestive physiology in pigs

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M.W.A. Verstegen, J. Huisman and L.A. den Hartog (Editors)

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PREFACE

This book provides a compilation of papers presented at the Vth Congress on Digestive Physiology in Pigs held in Wageningen (Doorwerth), The Netherlands.

Background and aim of this symposium are a consequence of changes in the field of digestive physiology of pigs.

During the last years many new advances have been made in the field of animal nutrition. New disciplines have been used to increase our knowledge on digestion and digestive processes. The symposium programme shows some of the new research fields in the area of digestive physiology in the pig. It is the Vth in a series of symposia aimed at discussing results of new studies and the development of concepts on digestive physiology in pigs.

In 1979, Dr. R. Braude and his co-workers at Shinfield, arranged the first international seminar on digestive physiology in the pig. The proceedings of this seminar can be found in the Technical Bulletin No. 3: "Current concepts of digestion and absorption in the pig" (Eds. A.G. Low and I. Partridge).

Three years later (1981) the second seminar took place at Versailles, arranged by Dr. A. Rerat and his staff. The seminar proceedings were published in "Les Colloques de l'INRA" no. 12 entitled "Digestive physiology in the pig" (Eds. J.P. Laplace, T. Corring and A. Rerat).

The third symposium was held in 1985 in Copenhagen. The proceedings have been published as "Proceedings of the 3rd International Seminar on Digestive Physiology in the Pig" 580, Beretning fra Statens Husdyrbrugsforsøg by the National Institute of Animal Science, Denmark (Eds. A. Just, H. Jørgensen and J.A. Fernandez).

The fourth symposium was held in Jablonna, Poland in 1988. The proceedings have been published by the Polish Academy of Sciences "The Institute of Animal Physiology and Nutrition, Poland (Eds. L. Buraczewska, S. Buraczewski, B. Pastuszewska and T. Zebrowska).

Papers in the Vth Symposium are written by many authoritive workers and are organised in five main topics concerned with various aspects of digestive physiology in pigs. In each session a first paper deals with a review of literature providing an update of the state of art of knowledge in this field. This provided an essential part of the symposium and enabled also a structured and a well organized discussion through its chairmen and discussion leaders.

We wish to acknowledge the financial support of the Vth Symposium by the organisation listed in the beginning of this book.

We would like to give special thanks to Mrs. J. van de Kraats-Bos, who provided such excellent secretarial help, essential for the preparation of the Congress and the proceedings.

SESSION 1

Animal factors affecting digestion and absorption

ANIMAL FACTORS AFFECTING PROTEIN DIGESTION AND ABSORPTION

A. RERAT[1] and T. CORRING[2]

(1) Unité sur l'absorption et le métabolisme hépatique
(2) Laboratoire d'Ecologie et de Physiologie du Système Digestif
CRJ - INRA, Jouy-en-Josas 78350 - FRANCE.

Abstract

Protein digestion and absorption is analysed in this review with a special attention paid to some essential parameters. Enzyme secretions at the various levels of the digestive tract are inventoried as well as their sequential action on proteins and peptides, their adaptability, neurohormonal regulation and animal factors such as age and nutritional and physiological status. The role of the various transport systems for aminoacids and peptides in the brush border and basolateral membrane of the enterocyte are examined as well as the nutritional consequences of these systems and the possible passage of native peptides into the portal blood. Amino acid metabolism in the enterocyte is analysed, in particular the metabolism of glutamine originating from the blood or lumen.

Introduction

The digestion of food depends on a variety of physiological parameters related with its physical and chemical characteristics and with other factors such as the rhythm of intake. The rate of food passage through the gut and intestinal enzyme capacities of the animal are of particular importance . Thus, the transit rate determines the time of contact between food and enzymes and this may be at the origin of a more or less complete hydrolysis of dietary constituents and the appearance of substrates inducing the development of some bacteria in the hindgut whose degradating action is not necessarily beneficial. The transit rate also regulates the time of contact between digestion products and absorptive surfaces; this may result in the formation of more or less voluminous residues despite a real but unfitted hydrolysis as well as in a delayed appearance of some digestion products in the "inner medium" leading to a poor metabolic utilisation (Elman, 1953). In the same way, any abnormal secretion or any absorption failure may lead to the presence of abnormally large amounts of nutrients in a segment of the digestive tract which they should not have reached with the same consequences as those of an inadequate transit. Hence, there is an interdependency between the various mechanisms of digestion in terms of motricity, secretion and absorption (Rerat et al., 1977a). The sequence of digestive events is therefore necessarily coordinated by the nervous and humoral relationships between the various organs. These relationships are characterized by the existence of retroactive systems which, at each step of digestion, are monitoring the importance and length of the previous and following steps. The regulatory peptides secreted by the mucosa throughout the digestive tract and by a variety of digestive organs such as the pancreas play a major role in the synchronization of the various digestive events.

These variables which are characteristic for the animal may be submitted to variations depending on the species, heredity, age, physiological stage (gestation, lactation, nutrition) and stress. They may also vary according to nutrients and type of feeding.

All the digestive events and their interrelations will not be analysed in this report. We have chosen to focus on protein digestion and to update our knowledge concerning:

- digestive hydrolyses en relation to enzyme productions in the various segments of the digestive tract, their adaptation and regulation;
- some intestinal transport mechanisms
- some aspects of enterocyte metabolism.

I. SEQUENCE OF DIGESTIVE HYDROLYSES, REGULATION AND ADAPTATION

From its entry into the mouth and during its progression through the gut, food is successively mixed with digestive secretions contributing to the sequential degradation of dietary macromolecules into nutrients which are subsequently absorbed. For proteins, these hydrolyses start in the stomach and continue in the intestine under the combined action of pancreatic and intestinal enzymes.

1.1. Gastric hydrolysis

The stomach secretes into the gastric lumen a complex mixture originating from various glands and mucosal cells, i.e. electrolytes, enzymes, mucosubstances, blood substances and other biologically active matters (such as the intrinsec factor). It also secretes a regulatory peptide, gastrin, into the blood circulation. The gastric juice is mainly characterized by two components, an acid component containing hydrochloric acid and water produced by the parietal cells and an alcaline component containing pepsinogens secreted by the chief cells or peptic cells as well as electrolytes such as chlore, carbonate, sodium and potassium secreted by the cardial glands also responsible for the production of mucosubstances.

The hydrochloric acid secretion has a variety of functions, i.e; activation of pepsinogens into pepsin, maintenance of intragastric pH at low values corresponding to the optimal pH for the action of pepsins; chemical digestion and stimulation of the duodenal production of cholecystokinin-pancreozymin.

Enzyme composition of the gastric juice

The gastric juice contains several pepsins and chymosin, i.e. proteases which initiate protein digestion. These enzymes are synthesized and secreted in the form of proenzymes converted into active enzymes by a limited proteolysis which separates a fraction of the N-terminal portion from the peptide chain (Kageyama & Kagahashi, 1980). This process triggered by hydrochloric acid is very rapid at pH 2 and goes on more slowly at pH 4 by autocatalysis due to the pepsin formed. In humans, distinction can be made between 7 pepsins distributed into two major groups according to their physicochemical characteristics, their activity and optimal pH of action (PGI:2.0 > pH > 1.5; PGII pH 3.2) (Samloff, 1971; Seijffers et al., 1965). In the pig, there are three (Foltmann, 1986) or four (Vonk & Western, 1984) pepsins, among which pepsin A is a major constituent with an optimum action at pH 2, pepsin B which is present in small amounts and not well known, pepsin C or gastricsin with an optimum action between pH 3 and 4. Young piglets (but not infants) possess another protease, chymosin (Foltmann et al., 1981) with the same optimum pH of action as that of pepsin C. However, gastric proteases are generally active within a large pH range and are all capable of coagulating milk at pH 6.5. The specific action of pepsins has been very well analysed (Taylor, 1968),i.e. endopeptidases which cleave the peptide bonds only between L-amino acids. The most rapid hydrolysis rate is observed for bonds near the aromatic amino acids, tyrosine and phenylalanine. The hydrolysis rate decreases for bonds involving glutamic acid and cystine and becomes very low for bonds between valine and glycine, tyrosine and cystine, tyrosine and serine. Glycylpeptides are very resistant to the action of pepsin.

Relationships between hydrolysis and gastric transit

Hydrolysis intensity depends on the length of stay of proteins within the gastric lumen and is thus responsible for the time length of contact between enzymes and substrates. This length of stay plays a major role for the first steps of food degradation since the pH of the fundic and pyloric contents changes with time, from 5 immediately after the meal to 2 after some time (Lawrence, 1972). Hence, protein hydrolysis is first slow and then more rapid. The gastric emptying rate can be reduced by various factors such as hyper- or hypo-osmolarity (Hunt, 1963) or an acid pH (Hunt & Knox, 1972). This emptying rate is controlled by receptors located in the duodenum which are sensitive to chyme acidity, osmolarity, levels of fatty acids and various amino acids: tryptophan in dogs and humans, phenylalanine, glutamate, arginine and cysteine in humans (Stephens et al., 1975; Byrne et al., 1977). Oligopeptides and proteins also participate as efficiently as their constituting amino acids in the reduction of the gastric emptying rate (Burn-Murdock et al., 1978; Stephens et al., 1976). The enzymatic hydrolysis of proteins depends on their amount and nature (Zebrowska, 1973) as well as on the nature of dietary lipids (Ziemlanski et al., 1972) and sugars (Buraczewski et al., 1971).

Gastric secretion pattern

The gastric secretions change with age. The secretion of hydrochloric acid may start at birth (Cranwell & Titchen, 1974), but seems to be delayed by the development of lactic acid fermentations in the stomach as affected by the environment at birth (Cranwell et al., 1976). The volume of gastric secretions increases rapidly during the first weeks of life and then more slowly (Noakes, 1972). There is no production of pepsin at birth while that of chymosin is rather high; after 2-5 days, the production of chymosin decreases and disappears after 4-5 weeks (Foltmann et al., 1981) while the production of pepsin increases first slowly then rapidly (Lewis et al., 1957; Decuypere et al., 1978)

Regulation of gastric secretion

Gastric secretion is monitored by a variety of nervous, endocrine and paracrine mechanisms. Gastrin secreted by the G cells of the gastric antrum as influenced by an antral distension (Soares et al., 1977) or by small peptides and amino acids (Richardson et al., 1976) represents one of the most important regulators of the secretion ; it is thus favoured by the ingestion of protein rich meals (Rerat et al., 1985). The two forms of this peptide, G17 and G34, act directly on the parietal cell and increase the acid secretion (Sachs et al., 1981). Gastrin may also act directly on the parietal cell via a local release of histamine (Main & Pearce, 1982). Gastrin secretion is modulated by another regulatory peptide, bombesin (Hirschowitz & Molina, 1983).

The nervous regulation of vagal origin is also an important control system of gastric secretion. This cholinergic-like vagal stimulation has been evidenced by the existence of a secretion during sham-feeding (Feldmann & Richardson, 1981). Acetylcholine and its analogues stimulate the secretion of pepsinogen both in intact animals (Samloff, 1971) and in isolated preparations (Kashekar et al., 1983; Sanders et al., 1983); this stimulation is inhibited by atropine which also inhibits the secretion induced by direct vagal stimulation (Hirschowitz, 1967).

The gastric secretion is also subjected to a paracrine control exerted by somatostatin. The somatostatin producing cells in the stomach are in a close contact with gastrin producing cells and with parietal cells (Larsson et al., 1979). Somatostatin strongly inhibits the secretion of gastrin and parietal cell secretions (Loud et al., 1985). Somatostatin is also stimulated by hydrochloric acid and its secretion by the gastric antrum and the parietal cell area is inhibited by the vagal nervous system (Larsson, 1980; Holst et al., 1983).Thus, the positive action of the

vagal nervous system may partly be due to the inhibition of the paracrine effect of somatostatin, which in turn inhibits the gastric secretion.

There is also a humoral and nervous regulation of gastric secretion of intestinal origin. The release of secretin into the bloodstream in response to the presence of hydrochloric acid in the duodenum causes a large inhibition of HCL production (Chey et al., 1981); it also inhibits gastric emptying (Vague & Andre, 1971) and seems to stimulate the in vivo secretion of pepsinogen (Stening et al., 1969), but this stimulatory action is being controversed. Somatostatin is also released by the pyloric antrum and the duodenum as affected by HCL and in addition to its paracrine action seems to behave as an inhibitory hormone (Larsson, 1980; Loud et al., 1985). The presence of nutrients in the duodenum inhibits the acid secretion, this inhibition being exerted together with several regulatory peptides whose secretion is stimulated by the nutrients, i.e. GIP (Pederson & Brown, 1972), neurotensin (Fletcher et al., 1985; Skov-Olsen et al., 1983), peptide YY (Adrian et al., 1985), enteroglucagon (Christiansen et al., 1976). Glucagon inhibits peptide and acid secretion related to meal ingestion in man (Konturek et al., 1975).

This variety of mechanisms intervene according to a given sequence at the moment of food intake. During the cephalic phase, i.e. when the meal is perceived or during sham-feeding, secretion of acid and of pepsin is stimulated by the pneumogastric. Thereafter, the arrival of food in the stomach induces the gastric phase of secretion triggered by distension of the organ and mainly the pyloric antrum and by the dietary protein content. The secretion is stimulated both via the excitation of the parasympathic fibres and by the release of gastrin, but also by the inhibition of somatostatin. The next step is the arrival of the food bolus in the duodenum which via physical (distension) and chemical stimuli (hydrochloric acid, protein nutrients) induces the intestinal phase characterized by an inhibition of gastric secretion, mainly acid, by various regulatory peptides secreted by the duodenal mucosa (GIP, neurotensin, secretin, peptide YY, somatostatin).

Consequences of gastric hydrolysis

Which is the result of gastric digestion ? *In vivo*, the nitrogenous matters are partly solubilized (up to 50% within 1h according to the nature of proteins), the soluble fraction being mainly composed of proteins, peptides and very few amino acids (Miranda & Pelissier, 1983; Low, 1979) and increasing with time after the meal. Hence, apart from the first emissions from the stomach, the gastric proteolysis is rather high. Protein hydrolysis is accompanied by a supply of endogenous proteins (6-8 g/day according to Cuperlovic et al., 1975; Zebrowska et al., 1975). No peptides or free amino acids seem to be absorbed from the gastric lumen (Rerat et al., 1988b).

The gastric phase of digestion can be considered as a preparatory phase and may be by-passed as shown by the consequences of gastrectomy on digestibility and absorption (Corring & Rerat, 1983).

1.2. RESPECTIVE ROLES OF POST-STOMACHAL HYDROLYSES

When the digesta arrive in the duodenum they are mixed with bile and pancreatic juice and as their pH value increases, their hydrolysis by pancreatic and intestinal enzymes starts and goes on while they are moving towards the distal segments. Digestion of proteins in the small intestine is very rapid and intense. It is due to the combined action of pancreatic enzymes responsible for endoluminal digestion and intestinal enzymes reponsible for membrane hydrolysis.

1.2.1. Exocrine pancreas enzymes

The pancreatic exocrine secretion of a wide spectrum of enzymes represents one major animal factor responsible for the enzymic degradation of many dietary

components in mammals. In the rat pancreas, twenty individual proteins have been identified by two-dimensional electrophoresis, i.e. four forms of procarboxypeptidase, three forms of trypsinogen, two or three forms of amylase, two forms each of chymotrypsinogen and proelastase, one form each of lipase and RNAse and four non identified forms of glycoproteins (Poort & Poort, 1981; Schick et al., 1984). Pancreatic proteolytic enzymes are secreted into the intestinal lumen in an inactive form (trypsinogen, chymotrypsinogen, procarboxypeptidases A and B and proelastase) Trypsinogen is activated by intestinal enterokinase and forms trypsin which in turn activates chymotrypsinogen, procarboxypeptidases A and B and proelastases (Keller, 1968; Rovery, 1988). A distinction is usually made between endopeptidases (trypsin, chymotrypsin and elastases) and exopeptidases (carboxypeptidases A and B). Other proteolytic enzymes such as collagenase and nucleases are present in the pancreatic juice. Endo- and exopeptidases cleave the inner and terminal peptide bonds of the protein molecule. Chymotrypsin and carboxypeptidase A cleave specific bonds near the aromatic L-amino acids (tyrosine, tryptophan, phenylalanine) while trypsin and carboxypeptidase B cut specific bonds near the basic L-amino acids (arginine and lysine). According to Gertler et al., (1980) the less specifically acting elastases as well as chymotrypsin play a major role in protein digestion. However, for optimal hydrolysis, all pancreatic proteolytic enzymes must act together.

Pancreatic enzyme deficiencies

In some diseases such as cystic fibrosis, which is a familial disease of the young, an almost complete loss of pancreatic enzyme activity is reported in 80 % of the patients suffering from a severe malnutrition. In animals, many experiments have shown that deprivation of pancreatic enzymes leads to a decrease in the apparent digestibility of energy and protein as well as in the absorption of dietary nitrogen. After total pancreatectomy, nitrogen absorption in dogs decreased by 30% one hour after the meal (Shingleton et al., 1955). In chickens (Ariyoshi et al., 1964) about 25% of ingested protein was used after pancreatectomy. In pigs, we found a 13% decrease in the apparent digestibility of nitrogen after the meal (Corring & Bourdon, 1977) and a 35 to 46% decrease in the appearance of free amino acids in the portal blood (Rerat et al., 1977b) when pancreatic juice was removed from the intestinal lumen. However, according to many data the exocrine pancreas possesses a large reserve capacity (Corring, 1980). In patients with chronic pancreatitis, maldigestion of dietary proteins was observed only when trypsin activity was under 10% of the normal (Di Magno et al., 1973) and the apparent digestibility of the diet was normal when only 1% of the pancreatic gland was kept in the rat (Uram et al., 1960). Moreover, the exocrine pancreas seems to be able to regenerate after a 95% pancreatectomy (Hotz et al., 1973). In the pancreatic duct-ligated pig, the apparent digestibility of nitrogen markedly decreased within the first 10 days, but increased significantly with time and the pigs grew in weight although to a lesser extent than intact animals. This improvement was the result of adaptative enzymatic increases reported by different authors (Senegas et al., 1976).

Pancreatic enzymes and food

It is well known today that pancreatic enzyme equipment can be markedly altered by diet composition. Long-term protein deficiencies cause severe disorders of the pancreas. Proteolytic enzyme activities are usually lower in the duodenal aspirates of infants when their diet is protein deficient. Dietary protein deprivation results in a loss of zymogen granules, shrinking and atrophy of the acinar cells and a reduction in the amount and duration of enzyme secretion from the pancreas. Following secretion into the small intestine, inactivation of pancreatic proteolytic enzymes increases when protein-free diets are fed. With total protein deprivation, the protein synthetic activity of the acinar cell corresponds almost exclusively to

the production of anionic proteases (trypsinogens 1 and 2, chymotrypsinogen 1, proelastase 1 and procarboxypeptidases A and B) (Schick et al., 1984). As these proteins represent almost 50% of the exocrine pancreatic enzymes in the rat, this adaptative process is a "last chance" for the cell and the body to get amino acid supplies for survival (Schick et al., 1984).

During protein malnutrition, is the disturbance of pancreatic digestive enzyme secretion large enough to exacerbate this malnutrition and impair refeeding ? When refeeding protein-deficient rats with a well balanced diet, the anabolism considerably increased and chymotrypsin activity reached higher values than those of the reference group (Keroua & Belleville, 1981). The exocrine pancreas modifies its enzyme secretion in response to diet composition (Corring, 1980). In animals, proteolytic enzyme activities either in the pancreatic tissue or juice entering the duodenum are modified by the dietary protein content while amylase and lipase activities, respectively depend on dietary carbohydrate and lipid contents. These variations are related to parallel increases or decreases in enzyme biosynthesis (Reboud et al., 1966; Poort & Poort, 1980). Five days to three weeks after administration of diets containing normal (22%) to high (82%) protein levels, the synthesis of most pancreatic proteins was directly proportional to the dietary nutritional substrate. However, chymotrypsinogen, anionic trypsinogen, proelastase 1 and procarboxypeptidases were the most markedly affected by the diet while the biosynthesis of trypsinogen 3, proelastase 2, RNAse and lipase were not modified (Poort & Poort, 1981; Schick et al., 1984). These findings indicate a differential effect of the diet on the biosynthesis of serine proteases and other proteolytic enzymes in terms of extent and timing of enzyme responses. Elastase gene expression only significantly increased after ingestion of a 70% protein diet while chymotrypsinogen and especially trypsinogen already increased after a 25% protein diet. Hence, the expression of pancreatic proteolytic genes was not altered by dietary protein to the same extent (Giorgi et al., 1985). According to Wicker et al., (1985) at least part of the adaptative regulation of protein synthesis in the rat pancreas is pretranslational either as a result of an enhanced transcription of the corresponding hydrolase genes or a reduced degradation of specific mRNA species.

The idea that there are minimal and maximal limits to secretion is clearly shown by lipase activities in response to growing amounts of dietary lipids (Sabb et al., 1986). Such limits in the adaptation have not been reported for other pancreatic enzymes. Amylase secretion is enhanced by increasing levels of starch intake (Noirot et al., 1981). Pancreatic proteolytic enzyme secretion is proportional or shows a "purposive" adaptative response to the amount of nitrogen intestinally infused in man (Vidon et al., 1978) or orally ingested by the rat (Schick et al., 1984). It also depends on the dietary protein quality (Valette et al., 1987) and no adaptative response was observed with protein of poor biological value (Johnson et al., 1977) or with a suboptimal supply of dietary protein (Temler et al., 1984).

Role of peptides in the nutritional regulation of pancreatic enzymes

It was formerly shown that in animals adapted to a diet enriched with a specific nutrient, the subsequent high level of this nutrient in the digestive tract induced an increase in the corresponding enzyme levels in the pancreas. It is interesting to note that it occurred without any contact between the nutrient and the pancreas. Ingested nutrients very rapidly undergo a physical and biochemical degradation which starts in the stomach. The stomach then empties a mixture of nutrients and hydrolysis products into the duodenum. Therefore, the information sent to the pancreas may be generated by the substrate or by its hydrolysis products. Several studies have been performed with an attempt to identify the product responsible for the pancreatic response and adaptation to the diet and the second messenger involved.

It is noteworthy that almost all experimental findings emphasize the effect of hydrolysis products on pancreatic secretion before their intestinal absorption

suggesting an active involvement of the intestinal mucosa via the release of the second messenger. Simoes Nunes & Corring (1980) reported that an intravenous injection of duodenal extracts from pigs fed on high-starch meals into recipient pigs elicited the secretion of pancreatic amylase. Simoes Nunes (1982) also showed in the pig that pancreatic adaptation to dietary carbohydrates and lipids was no longer observed after bypass of the proximal small intestine. In contrast, the pancreatic proteolytic enzyme adaptation to dietary proteins did not seem to depend on the proximal small intestine (Simoes-Nunes, personal communication). Bozkurt and Haberich (1985) reported that starch instillation into the duodenum led to a rapid decrease in amylase secretion whereas intraduodenal amino acids and lipids somewhat delayed the responses of proteolytic enzymes and lipase. The intestinal mucosa seems to be involved in the adaptation of the pancreas to the diet, but the mechanisms are probably different for each enzyme (Bozkurt & Haberich, 1985) and most likely involve many peptides. Only little information is available about the nervous control of the pancreatic adaptation to the diet. According to Morisset and Dunnigan (1967) this adaptation is not affected in vagotomized rats.

Among the regulatory peptides, cholecystokinin is often considered to be the main intestinal factor for pancreatic adaptation to the diet (Green et al., 1986). Stimulation with caerulein, a synthetic analogue of cholecystokinin, revealed both coordinate and anticoordinate rate changes (latency, kinetics and extent) in protein synthesis. Acute caerulein (25µg/kg/h) administred to rats decreased the secretion of amylase and increased that of anionic trypsinogens 1 and 2, chymotrypsinogens, procarboxypeptidases and RNAse within 3h, while cationic trypsinogen 3, elastase 2 and lipase synthesis were not modified (Schick et al., 1984). These findings are compatible with those observed after intake of protein-rich diets. However, according to Bozkurt and Haberich (1985) in rats and Langlois et al., (1989) in pigs, cholecystokinin does not have any specific effect on proteolytic enzymes, but rather a general stimulatory action on all pancreatic enzymes.

All peptides which are known to regulate the exocrine pancreas do not play an essential role in the adaptation of pancreatic secretion to the diet whereas currently unknown peptide-like components seem to be involved (Dick & Felber, 1975; Corring, 1977).

1.2.2. Intestinal enzymes

After hydrolysis caused by the sequential action of hydrochloric acid, gastric pepsins and pancreatic enzymes, mixtures composed of a large proportion (70%) of small peptides (2 - 6 amino acid residues) and free amino acids (30%) are very quickly released into the intestinal lumen (Adibi & Mercer, 1973; Chung et al., 1979). While the free amino acids are absorbed by specific transport systems, the peptides undergo a new hydrolysis before being absorbed by the digestive tract. The ultimate digestion of peptides is due to peptidases present in the brush border and the cytoplasm of the enterocytes of the intestinal villi. Because of these villi and the microvilli of the enterocyte apical membrane the intestine represents a very wide and a very efficient absorbant and digestive surface area (200 m^2 in man according to Wilson, 1962).

Intestinal peptidases

The brush border of the enterocyte contains a large amount of peptidases whose action is completed by other enzymes present in the cytoplasm. These enzymes are characterized by their specific activity. Thus, in the pig and rat the following enzymes have been identified in the brush-border membrane:

- an endopeptidase (Fulcher & Kenny, 1983; Kocna et al., 1980; Yoshioka et al., 1988) in small amounts (Danielsen et al., 1980) cleaving the peptide bonds near the

hydrophobic amino acids (Kerr & Kenny, 1974) inside oligopeptides and proteins such as casein (Guan et al., 1988)

- aminopeptidases cleaving neutral amino acids in the N-terminal position (NAP, neutral aminopeptidase; Maroux et al., 1973) or acid amino acids such as glutamic and aspartic acids (AAP, acid aminopeptidase, Benajiba and Maroux, 1980) or proline (aminopeptidase P; Lasch et al., 1986) or dipeptides, mainly X-Pro and X-Ala (Dipeptidyldipeptidase DPPIV, Svensson et al., 1978)

- one or several carboxypeptidases (Skovbjerg, 1981) releasing amino acids at the C-terminal position, mainly proline (carboxypeptidase P: Yoshioka et al., 1988; Erikson et al., 1989).

Furthermore, in the enterocyte brush border of humans and rats the following enzymes have been identified: folate conjuguase which separates the pteroylglutamic residue from folic acid (Reisenauer et al., 1977), glutathionedipeptidase which cleaves glutathion into its constitutive elements (Korak & Tate, 1982), dipeptidases mainly hydrolysing glycylleucine and aspartyllysine (Tobey et al., 1985) and glutamyl-transpeptidase (Hughey & Curthoys, 1976) which acts both as a peptidase and an exchanger of amino groups.

The action of these enzymes of which one fraction is released into the intestinal lumen as a consequence of mucosal desquamation (Andersen et al., 1988) is completed in the cytoplasm by that of several other peptidases:

- aminotripeptidase with a great affinity for tripeptides possessing a free α-amino group and those containing proline or hydroxyproline at the amino terminus (Adibi & Kim, 1981).

- several dipeptidases cleaving only dipeptides: glycylleucine dipeptidase with a very wide specificity, isolated in the pig (Noren et al., 1973), glycyl-glycine dipeptidase hydrolysing dipeptide Gly-Gly; tryptophan-alanine dipeptidase; prolyldipeptidase acting on Pro-X dipeptides (Noren et al., 1973); proline dipeptidase (prolidase) isolated in the pig (Sjöström et al., 1978) which is only active on aminoacylproline peptide bonds, i.e. on X-Pro dipeptides.

Other peptidases, probably present in cytosol, remain to be identified: leucine aminopeptidase, arginine aminopeptidase, pyroglutamate aminopeptidase and carnosinase (Sjöström and Nören, 1986)

Hydrolysis by intestinal enzymes

The hydrolysis of oligopeptides present in the intestinal lumen thus begins in the brush border of the microvilli and mainly concerns oligopeptides with more than three amino acid residues. Endopeptidase has probably only a minor role because of its analogy of function with pancreatic endopeptidases; its action leads to the formation of small-sized peptides. Aminopeptidase N separates a series of individual amino acids from the peptides, mainly leucine and methionine (Kim et al., 1976; Feracci et al., 1981). Aminopeptidase A separates glutamic and aspartic acids from the peptides, but has also a small activity towards arginine and N-terminal lysine (Danielsen et al., 1977). However, none of these two enzymes is able to separate proline in N-terminal position. When proline is in that position it can only be released by the joint action of two enzymes, i.e. dipeptidyldipeptidase which separates dipeptides of the aminoacylproline N-terminal type (Walter et al., 1980; Morita et al., 1983) in the brush border, and proline dipeptidase after transfer of these dipeptides in the enterocyte by a specific transport system. The magnitude of the carboxypeptidase action in the brush border has not yet been well established (Skovbjerg, 1981).

Thus, oligopeptides (C_2-C_6) present in the intestinal lumen are first broken down in the brush border into di-and tri-peptides and neutral and acid amino acids. A large fraction of tripeptides (60%) and a minor fraction of dipeptides (10%) are also hydrolysed at this level apart from those containing a proline residue (X-Pro or X-hydroxyproline). The remaining di and tri-peptides are transferred by a specific transport system into the cytoplasm (Adibi & Kim, 1981) where they are

hydrolysed to a large extent. However, part of them might not be hydrolysed and be conveyed in the native state into the portal blood (Gardner 1984).

Topographical variations

The amounts of peptidases found in the intestinal mucosa are topographically variable. They are inexistent in the crypts of the villi in which the enterocytes are still non differentiated and maximum in the medium and upper part of the villi (Nordstrom et al., 1968). The activity of some peptidases is larger in the rat ileum (DPPIV, NAP, AAP, carboxypeptidase) than in the proximal small intestine (Norén et al., 1980; Skovbjerg, 1981; Triadou et al., 1983); however, this is controversed for aminopeptidase N (Miura et al., 1983)The proximal-distal gradient is also observed for lysosomal enzymes in the young rat (Nikolaievskaia & Chernikov, 1986). The distribution between brush border peptidases and cytosol peptidases is variable according to intestinal segments, the former being dominant in the ileum and the others in the jejunum. According to Silk et al., (1976) this might signify that luminal peptidases play a more important role in the ileum than in the jejunum.

Regulation of peptidase activity

The aminopeptidase activity appears to be regulated by hydrolysis end products. The hydrophobic amino acids seem to inhibit the enzyme activity of the brush border (Kim & Brophy, 1979). Thus, alanine, methionine, leucine and histidine have a very marked inhibitory effect on a mixture of cytoplasm and membrane enzymes. Furthermore, various dietary factors may play an important role. In young rats, fasting caused a depression of brush border enzyme activity probably related with the absence of nutritive substrate, but it stimulated the cytosol enzyme activity most likely because of a higher protein turnover due to neoglucogenesis. The ingestion of casein rich meals stimulated not only the activity of brush border enzymes (Saito & Suda, 1975), but also that of cytosol enzymes apart from proline-leucine peptidase (Nicholson et al., 1974); these adaptative events were mainly marked in the ileum (McCarthy et al., 1980). The ratio between aminopeptidase N and isomaltase activity increased when the dietary protein content rose from 5 to 20% in the rat (King et al., 1983). Aminopeptidase activity increased in the brush border after administration of small peptides to the rat, but not after feeding a mixture of free amino acids (Fuse et al., 1989). On the other hand, aminopeptidase N activity decreased during total parenteral nutrition (Galluser et al., 1989). A deficit of valine led to a depression in leucine aminopeptidase activity (Kimura et al., 1978).

Factors of variation of peptidase activity

Some factors such as the age (Ugolev et al., 1979; Kedinger et al., 1986; Austic, 1985), physiological status (gestation, lactation; Rolls, 1975) or intestinal resection may lead to a variation in intestinal peptidase activity. This activity can generally be observed very early during foetal development of piglets. It is very high at birth but is momentaneously inhibited by colostrum ingestion which in the rat and pig contains a peptidase inhibitor (Lindbergh et al., 1975). Some peptidase activities then increase during the first weeks of life of piglets (Tivey & Smith, 1989) and thereafter decrease until a subnormal level at 8 weeks (Lindbergh & Carlsson, 1982). The pattern of peptidase activities is similar in the rat (Lindbergh & Owman, 1966); thus, they generally increase during late gestation, are maximum at birth and decrease within 3 weeks till the adult level; these activities are lower in suckled than in non suckled rats. Moreover, 2 weeks after birth there is a provisional rise in cytosol peptidase activity parallel to that of lysosomal cathepsins (Vaeth and Henning, 1982). This is interpreted as an adaptation to the absence of luminal protease, the milk proteins being absorbed by pinocytosis and

broken down by the successive action of lysosomal and cytosolic enzymes. In the rat the aminotripeptidase activity in the jejunum remains stationary for 2 weeks and thereafter increases (Noack et al., 1966).

After massive intestinal resection, aminopeptidase activity increases in large proportions when expressed by intestinal length unit, but not by DNA unit (Garrido et al., 1978). Accordingly, this increase only reflects the existence of a mucosal hyperplasia, but does not account for any change in the cell content. These facts have been confirmed for leucine aminopeptidase (Albert et al., 1990) and N aminopeptidase (Chaves et al., 1987) present in the ileum after a large proximal resection. After a pancreas shunt the intestinal secretions are also stimulated, as shown in the hamster (Senegas et al., 1976), and correspond to a weight increase of the intestinal mucosa (Corring & Bourdon, 1976).

2. TRANSPORT OF HYDROLYSIS PRODUCTS IN THE INTESTINAL CELL WALL

After the above described hydrolyses, a mixture of non digested proteins, small peptides and free amino acids reach the absorptive surface areas of the intestine. The extent and mechanism of transport are different for these substrates. Although many advances have been made the last few years in our knowledge of intestinal transport systems, there are still many gaps and less information is available than in the case of transport in other tissues (e.g. kidney, muscle). Data concerning the absorption of native proteins have been published in a recent review (Gardner, 1988) and will therefore not be analysed here.

2.1. Transport of amino acids

Amino acids are conveyed through the intestine by three systems i.e. an active, specific and saturable transport, a transport with a facilitated diffusion and a transport with a simple diffusion in the presence of high luminal concentrations of amino acids. The demonstration of these systems is difficult because of overlapping specificity and between-species differences. They can be ranked according to their sodium dependence, K_t determination from saturation kinetics, crossed inhibition profiles between pairs of amino acids and genetic deficiencies (Wellner & Meister, 1980) associated with malabsorption and often with urinary losses of a group of amino acids or of one amino acid. A supplementary parameter is based on the reaction towards rare amino acids conveyed by a single transport system and hence this system can be identified in other tissues and species (Hopfer, 1987). Almost all the information available in this field has been obtained in other species than the pig so that we have to use extrapolations for these animals. Conventionally (Bannai et al., 1984) each transport system is characterized by a letter or a group of letters indicating its specificity, expressed by capitals for the Na^+ dependent system and by small letters in the opposite case , with the exception of the L system which is not Na^+ dependent. Some of the transport systems identified in the intestine are responsible for the transport of amino acids across the brush border membrane and others across the basolateral membrane.

The brush border

- In the brush border, the neutral amino acids are mainly transported Na^+ dependently by a "public" system used by several amino acids (Christensen, 1984) and by three "private" systems. Most of them are conveyed by the NBB system (neutral brush border) whose characteristics are close to the L system found in other cell types, but different from those of the A system (Stevens et al., 1984). The catalytic constant K_t of this system is high (20 - 40 mmol/l). Methionine and phenylalanine are transported by the Na^+ dependent PHE system. Proline and hydroxyproline have a specific transport system (IMINO) excluding alanine and other short-chain amino acids and their transport capacity is high (Stevens et al.,

1984). The rabbit possesses a ß-system for transport of amino acids such as ß alanine (Munck et al., 1985).

- Cationic or basic amino acids (lysine, arginine, citrulline, ornithine) and cystine are transported by two "private" systems, one Na^+ dependent (Y^+) (Wolfram et al., 1984) and the other not (y^+) (the + sign indicate the necessity of a load on the lateral chain) (Stevens et al., 1984).

- Anionic or acid amino acids (glutamate, aspartate) are conveyed by a "private" Na^+ dependent system, $X_{G.A.}$ which is also responsible for the reverse transport owing to potassium or protons (Berteloot, 1984). The catalytic constant of this system is low (0.5 mmol/l).

The brush border also possesses an Na^+ dependent facilitated diffusion system which only represents a small fraction of the absorption (Stevens et al., 1984). This system has the same characteristics as the L system found in many other cells; its catalytic constant is low (K_t<1mmol/l). A second Na^+ independent system makes possible the absorption of ß-amino acids (Lerner, 1984).

The basolateral plasma membrane

The basal cell pole also exhibits routes of transport through the basolateral plasma membrane. The largest transport is made by the Na^+ dependent L system which is highly specific for neutral amino acids together with cysteine and glutamine (Stevens et al., 1984; Taylor et al., 1989). There are several Na^+ independent systems, one (asc) responsible for transport of neutral amino acids with 3-4 carbons , alanine, serine and cysteine (Lash & Jones, 1984), one of low capacity (y^+) enabling the release of basic amino acids and another that of proline (Davies et al., 1987).

An active Na^+ dependent transport has also been observed with low substrate concentrations (Mircheff et al., 1980); it is imputed to the presence of the A system in the basolateral membrane which transports short-chain polar amino acids and the ASC system which takes up neutral amino acids with 3-4 carbons,i.e. alanine, serine and cysteine. This might explain the uptake of amino acids from the blood by the enterocyte for metabolic purposes in the case of low intestinal absorption.

A special emphasis should be laid on the uptake of glutamic acid and glutamine by the basolateral membrane as these amino acids play an important role, one in the transaminations and the other as an essential energy source for the enterocyte. These amino acids accumulate in the isolated intestinal cell (Bradford & Mac Givan, 1982) or in intact epithelial cell preparations (Boyd and Perring, 1981) much more than in the case of neutral amino acids. Although it has not yet been demonstrated, the basolateral membrane most likely possess an Na^+ and K^+ dependent transport system similar to that existing in the brush border membrane or in the hepatocyte (Ghishan et al., 1990).

All these systems are responsible for the transport of all free amino acids from the intestinal lumen to the fluids irrigating the intestinal tissues. It is, however difficult to establish the respective role played by each type of transport (active transport, facilitated diffusion, simple diffusion) in the absorption processes because their interventions vary according to substrate concentrations.

Factors of variation

The transport capacities of the intestine change throughout the digestive tract as well as with age. They are much lower in the large bowel than in the small bowel; thus, the transport of methionine is high in the piglet colon at birth, but becomes very low within a few days (James & Smith, 1976). The transport of amino acids in the small intestine exists before birth in rabbits (Deren et al., 1965; Guandilini & Rubino, 1982) and guinea pigs (Butt & Wilson, 1968) and before hatching in chickens (Pratt & Terner, 1971). In these three species, its efficiency increases until the postnatal period. It decreases 2 or 3 days after birth and then

reach the adult level; this has been confirmed in the rat (Fitzgerald et al., 1971). There are interspecific differences in the range of changes and amino acids involved. As the catalytic constant K_t is not modified during this evolution of transport with age (Austic, 1985), it may be assumed that it is the number and not the type of transport systems which changes with time, but there are exceptions (Penzes & Boross, 1974)

2.2. Transport of peptides

2.2.1. Transport in the enterocyte

It was long considered that the free amino acids constituted the only form of appearance of protein digestion products in the portal vein (Van Slyke & Meyer, 1972). And yet, it was shown that the amounts of free amino acids appearing in the portal blood were often much lower than those disappearing from the intestinal lumen (Dawson & Porter, 1962). This difference was imputed to the metabolism in the gut wall of a fraction of absorbed amino acids since the presence of small peptides in the portal blood had not yet been demonstrated.

Demonstration of oligopeptide transport

Owing to technical improvements it has been shown *in vitro* and *in vivo* (Newey.& Smyth, 1959, 1960) that the form of hydrolysis products entering the enterocyte may be different from the form appearing in the serous part of the cell or in the mesenteric blood. In other words, it was demonstrated that small peptides could enter the intestinal cell without being previously hydrolysed and released in the portal blood in the form of amino acids. Oligopeptides disappear more rapidly from the intestinal lumen than mixtures of analogous free amino acids (Adibi & Philipps, 1968; Matthews et al., 1968). Besides, humans suffering from subtotal incapacities for the absorption of basic free amino acids (cystinuria) or neutral amino acids (Hartnup's disease) easily use mixtures of dipeptides containing these amino acids (Asatoor et al., 1970; Hellier et al., 1972) The numerous studies made on this topic have been reviewed (Matthews & Adibi, 1976; Adibi & Kim, 1981; Grimble et al., 1989). Hence, it is now generally admitted that peptides can be absorbed more rapidly in the brush border than free amino acids and that because of the presence of di and tripeptidases in the cytosol of the enterocyte, they are released towards the portal vein in the form of free amino acids. However, it was recently shown that in addition to the large quantities of amino acids appearing in the portal blood, there are rather substantial and variable amounts of small peptides which may be absorbed in the native form (Webb, 1986)

The specific transport of oligopeptides against a concentration gradient, owing to a system which requires energy, has been evidenced both by the absence of competition between peptides and free amino acids (Rubino et al., 1971) and by obtaining the inhibition of brush border peptidases *in vitro* by hypoxia (Ugolev et al., 1990; Smithson & Gray, 1977) or *in vivo* using various substances. By the latter method it was shown that the small peptides disappear from the digestive lumen without being hydrolysed, but apparently not the tetrapeptides (Adibi & Morse, 1977).

Biochemical factors of variation

The affinity of peptides for the transport system depends on their molecular structure. Thus, the number of amino acids belonging to the structure of the peptides play an important role. Hence, when the number of amino acids increases from 2 to 3, the K_t increases and the affinity of the peptide for the transport system decreases which results in a limitation of the transport (Adibi et al., 1975). The stereoisomeric form of each peptide represents another factor of variation of

peptide affinity towards its transport system. The absorption rate strongly decreases when amino acids of the L-form are replaced by D-forms, the decrease being less pronounced when there is only one amino acid of the D-form (Asatoor et al., 1973). In fact, D and L enantiomorphs of the dipeptides use the same transport system with a lower affinity for the D forms.

The lateral chain length constitutes a major factor liable to change the affinity of the peptide for the transport system. An amino acid with a longer lateral chain such as leucine provides the peptide with a higher affinity for the sites of membrane absorption (Adibi & Soleimanpour, 1974). It seems that sometimes its position, carboxy-terminal or amino-terminal, may have a different influence. Thus, lysine is absorbed more rapidly when placed in the N terminal position in a dipeptide with glycine than when it is placed in the C terminal position. The opposite is observed when it forms a dipeptide with glutamic acid (Burston et al., 1972).

Other characteristics may be involved. Thus, peptides containing basic or acid amino acids have a lower affinity for the transport system than peptides containing neutral amino acids (Addison et al., 1975).

A unique intestinal transport system for peptides

In contrast to amino acids, di- and tripeptides have only a single active transport system. This system has a very high specificity since it is indifferent to the electrical load of the lateral chain of the amino acids and accept all peptides whether they contain neutral, basic or acid amino acids. Its activity can be allosterically modified by the most hydrophobic peptides (Matthews & Burston, 1984; Rajendran et al., 1985). The peptides are competing for this common transport system (Matthews et al., 1975; Taylor et al., 1980). Thus, methionylmethionine is a potent inhibitor of carnosine absorption (Addison et al., 1974) . In contrast, there may be stimulatory effects as in the case of glutaminylglutamate for carnosine (Addison et al., 1974). *In vivo*, there is a slowing down of peptide absorption in the presence of carbohydrates in the pig (Rerat et al., 1990).

Driving force of peptide transport

Although opposite arguments have been supplied by some authors (Matthews et al., 1979), the transport of peptides seems to be Na^+ independent (Ganapathy et al., 1981). The driving force of peptide transport seems to be constituted of an electrochemical proton gradient (Ganapathy & Leibach, 1985). It was thus shown that the accumulation of peptides in the membrane vesicles of the brush border took place in the presence of an H^+ gradient (Takuwa et al., 1985; Ganapathy et al., 1987).

In addition to the active transport responsible for the absorption of peptides by the enterocyte against a concentration gradient, there is a simple difffusion system which is little involved at low concentrations, but which plays a major role at high concentrations.

Topographical and physiological variations

In the rat, the transport of peptides is generally more marked in the jejunum than in the ileum (Crampton et al., 1973). In the rabbit, (Guandilini & Rubino, 1982) the rate of uptake (glycylproline) increases from mid-gestation, culminates at birth and then decreases until the adult level.

2.2.2. Transport of peptides in the portal blood

Determination of peptides in the blood

Most peptides are hydrolysed by cytosol peptidases in the enterocyte while some of them are not broken down but released as native petides into the mesenteric vein. This was long ignored because of technical failures in the assessment of peptides in the blood. Folin and Berglund (1922) were the first to make such assumptions; they showed that there was a large increase in the concentration of non protein, non urea, non amino nitrogen in the blood of humans fed with gelatine. The results of Dent and Schilling (1949) on the basis of jugular and portal plasma concentrations of peptides after the meal in the dog showed in agreement with Christensen (1949) that at least a fraction of proteins was absorbed as peptides. The appearance of dipeptides in the blood plasma during digestion has been clearly evidenced in particular cases such as that of an artificial dipeptide, glycyl-glycine (Adibi, 1971), peptides resistant to hydrolysis by dipeptidases as those which contain hydroxyproline and which are present in gelatine (Prockop et al., 1962) or carnosine and anserine, present in chicken breast proteins (Perry et al., 1967). The appearance of peptides on the serous side of the intestine has also been shown *in vitro* during perfusion of intestinal loops isolated by means of enzyme proteolysates (Gardner et al., 1982). *In vivo* it was claimed in the hamster that the proportion of amino nitrogen appearing in the portal blood in the form of peptides after ingestion of a casein proteolysate ranged around 10% (Gardner, 1984), but no direct evidence for this absorption has been supplied. In the calf, the size of the peptides appearing in the portal vein has been estimated (Schlagheck & Webb, 1984) and their proportion found to represent 70% of the amino nitrogen appearing in the portal vein. In contrast, quantitative studies performed *in vivo* in the pig using enzyme hydrolysates of milk proteins showed that the rate of appearance of amino acids in the portal vein reached 100%; hence there was no possibility for the appearance of native peptides in the portal blood and according to the analyses, no peptides were found (Rerat et al., 1988a). These discrepancies clearly show the necessity of developing well fitted techniques of analysis for determining the amounts of peptides absorbed in the native form as well as their size and type and the conditions which favour their absorption. In the present state of knowledge it is impossible to estimate the range of peptide appearance in the portal blood. Such an estimation would have interesting consequences for some types of physiologically active peptides (Gardner, 1984).

Fate of peptides appearing in the portal blood

Which is the origin and which is the fate of these blood peptides ? They may originate from the gut lumen, but they may also be degradation products of intestinal protein metabolism. Peptides appearing in the blood cannot be hydrolysed in the plasma because its hydrolase activity is low or inexistent, as shown for glycylglycine and glycyl-leucine (Krzysik & Adibi, 1977). In contrast, dipeptides are easily used by the tissues since after intravenous administration of glycyl-leucine it rapidly disappeared from the blood plasma, but was not found in the urine or in the intracellular spaces of various tissues (Adibi et al., 1977). The tissues exhibit a marked hydrolase activity towards peptides whether they are exogenous or originating from tissue protein degradation with a large proportion of di- and tripeptides. Peptides are thus hydrolysed in the intracellular compartment during a very rapid process so that the existence of an intracellular pool of peptides has only been demonstrated by means of a specific peptidase inhibitor, bestatine (Botbol & Scornick, 1983). Evidence for an intracellular hydrolysis of exogenous peptides followed by the incorporation of their constitutive amino acids

into the tissue proteins has been obtained by a comparative injection of ^{14}C glycine or ^{14}C glycyl-glycine (Krzysik & Adibi, 1979) the labelling of tissue proteins being generally the same in both cases.

2.3. Adaptation of the intestinal transport

The absorption of nutrients by the samll intestine varies according to a variety of natural factors such as the physiological status (lactation, gestation) or diet changes and according to experimental factors such as intestinal resection. The events involved can be divided into two categories, either a rise of the non specific overall absorption capacities, i.e. concerning all nutrients and related to an increase in the absorptive surface area, or the induction or repression of specific transport mechanisms, variable according to the availability of transported substrate or their stock in the body.

Non specific adaptation

The most striking examples of non specific adaptation concern the intestinal resection and the lactation status. Intestinal resection reduces the absorptive area and causes a decrease in the time of passage of food through the digestive tract. However, when the resection involved less than half the digestive tract, no malabsorption phenomena were observed in the rat, dog or man (Young & Weser, 1974; Weser, 1979). This was also the case for pigs subjected to enterectomy of the proximal or distal small intestine (Laplace, 1976). Hence, there was a compensatory absorption in the remaining intestine, the mechanism of which has been studied mainly in the pig, but also in the rat subjected to resection of the proximal small bowel. The absorption per cm of intestine increased for all nutrients whether they were amino acids and peptides (Garridio et al., 1978; Menge et al., 1981) or glucose (Dowling and Booth, 1967). This resulted from an increase in the surface area per cm of intestine due to an increment in the height of the villi and in the number of enterocytes per villus (Menge et al., 1983), but without any increase in the capacity of each enterocyte for the transport of amino acids. A similar adaptative mechanism can be observed in lactating females whose feed intake increases in large proportions during this period. In lactating rats, the digestibility of the diet was thus maintained (Fell, 1972); glucose and amino acids were absorbed more rapidly than in control animals (Cripps & Williams, 1975), proline exhibiting the opposite trend (Datta & Sharma, 1985). Non specific adaptative phenomena of the same kind can also be observed during other types of hyperphagia such as after exposure to cold (Jacobs et al., 1975) or after destruction of the ventromedian hypothalamic nuclei (Brobeck et al., 1943).

Specific adaptation

The specific adaptation concerns the change in the absorption of a single nutrient subsequently to a change in the dietary supply of this nutrient, and this may lead to a response in the same direction as the change in the supply or in the opposite direction (Diamond & Karasov, 1987; Obata et al., 1989). Thus, the amino acid absorption ability of the intestine increases both after adaptation to high and low levels of protein intake (Christensen, 1984). A comparative study of the intestinal absorptive capacities using everted intestinal rings after adaptation to two diets, either rich in carbohydrates with a moderate casein content (15%) or poor in carbohydrates and rich in casein (75%), showed that the uptake of proline was much higher with 75% than with 15% proteins and the opposite occurred with glucose (Karasov et al., 1983). For each substrate and each change in the dietary regimen, the stimulation of the transport is completed in one day while its inhibition requires several days . Thus, a diet switch causes an acceleration in the uptake of a nutrient (proline) and a reduction in the uptake of another nutrient

(glucose) according to different schedules. Accordingly, the specific intestinal transport systems undergo an induction or a repression caused by the level of dietary substrates. Taking into account that the intestinal mucosa has a biological half-life of 17-18 h, these changes can either be due to the induction of new transporting molecules in existing cells or to the production of new cells rich in these molecules. The influence of feeding on the transport of amino acids has been widely demonstrated. In the rat, fasting or feed restriction stimulated the transport of various amino acids (Lis et al., 1972; Hindmarsh et al., 1967) The incorporation of high levels of proteins to the diet stimulated more or less the transport of amino acids in the rat (Scharrer & Brugemann, 1971). Conversely, a low protein level reduced the transport of some amino acids, but increased that of others (Wapnir & Lifshitz, 1974). The transport of methionine increased after intake of methionine rich diets (Lis et al., 1973) while the intake of an excessive amount of phenylalanine brought about a decrease in the transport of aromatic amino acids (Wapnir et al., 1972).

There are also examples of a hormonal regulation of the intestinal transport of amino acids, involving prolactin, somatotropin and thyroid hormones (Alpers, 1987).

2.4. Nutritional consequences of specific transport systems in the intestine

Kinetics of appearance of free amino acids in the portal vein

According to present data, the systems of intestinal transport of peptides are independent of those used by the free amino acids and hence it may be assumed that the competition for the sites of transport is reduced and the absorption more efficient. However, only few experiments have been carried out on this topic *in vivo*. The criteria used were either the rate of disappearance of nitrogenous matters from the intestinal lumen of perfused intestinal loops (Silk et al., 1982) or the level of appearance of α-amino nitrogen in the peripheral blood (Silk, 1976). The spatio-temporal capacities for digestive compensation of the gastro-intestinal tract cannot be expressed by such approaches. Moreover, the intensity of absorption is very poorly perceived, the variations in the systemic blood being buffered by the uptake of amino acids by the liver. These shortcomings are avoided by a method of *in vivo* measurement of the appearance of amino acids in the portal vein and their metabolism in the intestinal wall and the hepatic tissue (Rerat, 1988). This method was used in animals fitted with a permanent cannula in the duodenum and which received duodenal infusions of solutions of small peptides obtained by mild enzymatic hydrolysis of milk proteins or solutions of free amino acids of the same composition (Rerat et al., 1985, 1988a). Under these conditions, the appearance of amino acids in the portal blood occurred very early since 66 to 68% of the total amounts absorbed within 5h were absorbed during the first 2h, whatever the type of proteins supplied. During the 5h of observation, the quantities of total amino acids absorbed were significantly higher after infusion of oligopeptides than after infusion of free amino acids. (Absorption coefficient for 106g perfused: 101 vs 58%).

The change with time in the absorption of most of the individual amino acids resembled that of their sum. Thus, the appearance of amino acids in the portal blood was greater, more rapid and more homogeneous after duodenal infusion of a solution of small peptides than after infusion of a solution of free amino acids, irrespective of the amount of the perfusate. The coefficient of "absorption" of most individual amino acids was higher after infusion of a solution of small peptides than after infusion of a solution of free amino acids, irrespective of the time elapsed after the infusion. However, there were some exceptions. Thus, methionine exhibited a significantly higher absorption coefficient after infusion with free amino acids and the absorption coefficients of isoleucine, aspartic acid + asparagine and glutamic acid + glutamine were equivalent for the two types of infusions. Even when absent from the infusates, some amino acids such as asparagine, ornithine

and citrulline appeared in rather large quantities after infusion of both types of solutions.

Thus, the physico-chemical nature of the solution had a marked influence on the hierarchy of absorption of individual amino acids.

Comparison with the disappearance of amino acids and peptides present in the intestinal lumen

The data obtained, especially those concerning the appearance of individual amino acids in the portal blood, may be compared to those derived from other techniques by means of which the rate of disappearance from the digestive lumen was measured by oro-duodeno-jejunal intubations in man (Nixon and Mawer, 1970) or isolated intestinal Thiry-Vella loops *in vivo* in man (Orten, 1963) or isolated loops *in situ* in the rat (Delhumeau et al., 1962). The hierarchy of appearance of free amino acid mixtures in the portal blood was usually similar to that of their disappearance from isolated loops. Among essential amino acids, methionine and branched chain amino acids were absorbed most rapidly in both cases and lysine, histidine and threonine more slowly. Among the non-essential amino acids, diacids were the slowest to be absorbed. There are discrepancies between these data and the rate of disappearance from the digestive lumen of some amino acids synthetized in the gut wall (low for glycine and very low for alanine) and their very high rate of appearance in the portal blood. However, it is generally admitted (Sepuldeva & Smith, 1978, 1979) that hydrophilic neutral amino acids of low molecular weight are conveyed less easily than hydrophobic amino acids of higher molecular weight, the transport of basic amino acids being intermediate.

During nutrition with free amino acid solutions, this competition for absorption sites may be responsible for a change in the composition of the amino acid mixtures available to the body. However, these consequences of competition might be reduced (Christensen, 1963) since absorption occurs throughout the digestive tract. Hence, amino acids with a high affinity for the transport systems can be absorbed in the proximal intestine and those with a lower affinity in the more distal part of the small intestine where competition is no longer operative. Our results showed that the mixture of ingested amino acids was partly modified during its transport and this was more marked with non-essential than with essential amino acids. The spatio-temporal compensation is therefore only partly involved in the reduction of competition between amino acids when they are administered in the free form.

After peptide infusion, the hierarchy of absorption is greatly modified the highest rate being that of aromatic and basic amino acids and the lowest one that of methionine. The more rapid absorption of amino acids after duodenal infusion of oligopeptides than after infusion of free amino acids and the modification of the composition of the absorbed mixture may be explained by different competitive processes linked to the existence of specific sites of transport for di- and tripeptides in the intestinal wall (Matthews, 1972; Matthews and Adibi, 1976). This hierarchy of amino acid absorption is similar to that found in man using duodeno-jejunal intubations for determining the kinetics of disappearance of amino acids from the intestinal lumen (Crampton et al., 1971; Silk et al., 1973), but these authors used hydrolysates containing large amounts of free amino acids leading to interferences with the absorption. The rate of disappearance from the intestinal lumen increased with decreasing size of peptides (Silk et al., 1985). Hence, di- and tripeptides were more rapidly absorbed than tetra- and pentapeptides (Rees et al., 1987), the hydrolysis being thus considered as the limiting factor (Grimble et al., 1987). It would be interesting to determine why the transport of methionine was slowed down when complex partial hydrolysates were used. Indeed, during *in vitro* experiments using methionine containing dipeptides, the transport of this amino acid was facilitated as compared to that of free methionine (Crampton et al., 1973). Is it the size of the methionine containing peptides or their lower affinity for the sites of transport for which they compete with other small peptides which are

responsible for this slowing down of the transport of méthionine by the intestinal wall when partial hydrolysates are used ? The improvement of present knowledge of the size distribution of small peptides of hydrolysates by a more accurate analysis of their amino acid content will contribute to answering such a question as well as that concerning the possibility of more general types of competition between small peptides and the existence of transport groups.

Nutritional consequences

What can we deduce from these competitions between amino acids and possibly between small peptides under natural feeding conditions ? It is known that during digestion of serum albumin proteins (Adibi and Mercer, 1973) hydrolysis of proteins in the duodenum results in the appearance of large concentrations of small peptides in the duodenum, 3 to 6 times higher than those of free amino acids. This was also found in the ileum, but to a lesser extent. Moreover, it appeared that the relative proportions of these two groups of nitrogenous substances varied mainly with the protein ingested (Zebrowska, 1973). Because of the large number of factors involved (proportions and composition of the mixture of free amino acids, proportions of the mixture of small peptides, size and amino acid composition of each of them, variable rate of absorption for each of the components of these mixtures depending on their competition), the hierarchy of absorption cannot be the same from one protein to another unless there are spatio-temporal compensations. In fact, the hierarchy of disappearance of amino acids from the digestive lumen throughout the small intestine was not the same for casein as for a barley-soyabean based diet (Zebrowska, 1981); similarly, the hierarchy of amino acids appearing in the portal vein varied from one protein to another (Rerat, 1988).

The consequences of this double transport system involving, on the one hand, the amino acids and, on the other hand, the peptides are as follows:

- a very rapid disappearance of the proteins after meal intake. According to Fisher (1954) a complete hydrolysis of proteins *in vitro* requires 36-155h and even when using more modern methods for the dialysis of released products and a better analogy between *in vitro* and *in vivo* digestion (Galibois et al., 1989), the time needed for complete hydrolysis remains very long. It may therefore be assumed that the very rapid absorption of protein digestion products is never preceded *in vivo* by a complete hydrolysis (Fisher, 1954).
- during genetic failures of certain amino acid transport systems (Hartnup's disease) no consequences of protein malnutrition can be observed because of the absorption of amino acids in the form of peptides in the enterocyte.
- in enteral nutrition, the kinetic advantage provided by the peptides is obviously very interesting in the case of partial resections of the digestive tract because the faster absorption of peptide solutions leads to a maximum utilisation of the residual intestinal areas.
- in terms of metabolism, the utilisation of peptides in enteral nutrition may prove to be interesting because the absorbed amino acid mixture is more homogeneous and their rate of appearance higher in the portal blood. In pigs fitted with permanent cannulas of the portal vein, carotid artery and hepatic vein as well as with flowmeter probes around the portal vein and hepatic artery, it was possible to establish hepatic and peripheral balances . Thus the profile and amount of the amino acid mixture taken up by the liver and by the tissues after duodenal infusion of small peptides was different from that observed after infusion of a mixture of free amino acids of the same composition. The mixtures of amino acids retained by the peripheral tissues was more imbalanced after infusion of free amino acids and correlatively, the production of hepatic urea was higher (Rerat, 1988). The biological value of these peptide mixtures was higher in the rat (Monchi et al., 1991) than that of free aminoacid mixture.

3. Metabolism of amino acids in the enterocyte

During the postprandial period, a large flux of amino acids of luminal origin reaches the enterocyte and decreases within a few hours in the absence of a new meal; it is thereafter replaced by another flux from the blood. The amino acids undergo various changes in the intestinal cell. They may either be metabolized or incorporated into proteins or released as such into the portal blood. Their metabolism involves the synthesis of non-essential amino acids, regulatory peptides and pigments; they may also be catabolised by transamination ou deamination and constitute a source of organic acids and energy. Their intracellular pool only remains constant because of the balance between metabolism and de novo synthesis of amino acids and between synthesis and degradation of intracellular proteins.

3.1. Protein synthesis

The uptake of amino acids of luminal origin has been known for a long time. Their *in vitro* incorporation into intramucosal proteins ranges around 10% (Bronk et Parsons, 1966). They seemed to be more directly used for the synthesis of intestinal proteins than amino acids derived from the blood. (Hirschfield et Kern, 1969). The latter seemed to be preferentially incorporated into crypt cell proteins and the amino acids of luminal origin into the cells of the villi (Alpers, 1972). The utilisation of amino acids of luminal origin for the synthesis of enterocyte proteins was particularly evident during prolonged parenteral nutrition which caused a mucosal atrophy (Levine et al., 1974*b*) or after intestinal resections which only led to a compensatory hyperplasia in the case of enteral nutrition (Levine et al., 1974*a*).

In vivo, the importance of this uptake of amino acids of luminal origin is illustrated by the difference between the total amounts of amino acids appearing in the portal blood and the amounts ingested in pigs receiving large meals after a previous fasting (Rerat, 1988). When digestion was finished, i.e. when the porto-arterial differences disappeared, this difference represented 30 to 38% of the amount of protein ingested in pigs fed large wheat or barley meals and even more for carbohydrates. A very large fraction of ingested proteins was thus not recovered as amino nitrogen in the efferent blood of the intestine. This absence does not necessarily signify an uptake, but may be due to faecal excretion. However, the difference between amounts disappearing from the proximal gut and amounts ingested only represented 19 and 25%, respectively for wheat and barley. The amounts of amino nitrogen disappearing from the digestive tract were thus 10 to 15 points higher than those appearing in the portal vein. Furthermore, as the balance of the exchanges between arterial blood and intestinal lumen of the other forms of nitrogen, urea and ammonia nitrogen, leads to a larger diffusion of urea nitrogen towards the intestinal lumen than the corresponding appearance of ammonia in the portal blood (Rerat & Buraczewska, 1986), the deficit of amino nitrogen appearing in the portal vein relative to the amounts ingested can only be explained by an uptake by the intestinal tissues.

The metabolism of proteins in the intestinal tissues is very active. These tissues exhibit the highest fractionary synthesis rate in the body (Garlick, 1980), as evidenced also by the rate of enzyme induction in the villi (Rosensweig et al., 1971) and by the very high rate of endogenous amino nitrogen turnover (Alpers et Kinzic, 1973). Despite this synthesis the total protein content of the intestinal tissues and that of the cells remain constant. An intense desquamation takes place throughout the digestive tract which corresponds to protein losses in the intestinal lumen compensated by the synthesis of new cells. A degradation of the proteins formed may occur in the cells, mainly in the brush border enzymes (Kaufman et al., 1980). There may also be a secretion of proteins towards the intestinal fluids. This secretion mainly concerns some plasma proteins such as apolipoproteins A_1 and

A_{IV} (Glickman et Green, 1977; Magun et al., 1985 ; Hollanders et al., 1985 ; Green et al., 1980) ;it is often highly stimulated by the ingestion of lipids. Other apolipoproteins are very poorly secreted (Blaufuss et al., 1984). There are most likely other proteins which are secreted into the circulating fluids but they have not yet been evidenced apart from alkaline phosphatase (Young et al., 1981).

3.2. Amino acid metabolism.

Aminoacids of luminal origin

While it is rather easy to measure the appearance of amino acids in the portal vein and *a contrario* to deduce the uptake of dietary nitrogen by the intestinal tissues during late digestion, it is much more difficult to assess the final uptake of luminal individual amino acids. This is due to their variable kinetics of absorption and hence the end of the digestive processes cannot be accurately determined even during long-lasting experiments. However, it is possible to deduce the magnitude of intestinal metabolism in two situations: excess of amounts appearing in the portal blood relative to amounts ingested and identification in the portal blood of amino acids absent from the diet. In addition, it is also possible to measure the apparent uptake of blood amino acids by the intestinal tissues on the basis of the portoarterial differences which are negative during the post-prandial period when the arterial concentration of the amino acid exceeds the portal concentration. Measurement of intestinal blood input (arterial flux) and output (portal flux) of amino acids after intestinal infusion of small peptides or amino acids of the same composition clearly shows this variable metabolisation in the gut wall according to the amino acid considered (Rerat, 1988) and the physico-chemical nature of the infusate. This metabolisation is particularly evident for certain non-essential amino acids such as alanine and to a lesser extent glycine exhibiting larger amounts in the portal blood than the amounts infused. It is also evident for citrulline and ornithine absent from the diet and appearing in rather large amounts in the portal vein. These amounts widely exceed those of arterial origin taken up by the intestine. In contrast, the very low rate of appearance of diacids and mainly glutamic acid in the portal blood (11-23%) can only be explained by the involvment of this amino acid in the reactions of deamination and transamination so that it partly disappears from the absorption balances (Neame & Wiseman, 1957, 1958 ; Pion et al., 1964)

Amino acids of arterial origin

A large apparent uptake of amino acids of arterial origin has also been observed. It was higher after duodenal infusion of free amino acids than after infusion of small peptides (Rerat, 1988). The differences in favour of the infusion of free amino acids were significant for total amino acids (34g vs 25g/8h) and essential amino acids (11g vs 6g/8h) ; they were particularly marked for lysine et branched-chain amino acids whose uptake by the intestine was four times higher after infusion of the solution of free amino acids. In both cases there was a large uptake of glutamine, representing 40% of total non-essential amino acids and a smaller uptake of glutamic acid and proline. This might be explained by the always higher concentration of circulating free amino acids after duodenal infusion of the solution of free amino acids than after that of small peptides; hence, they can then be used in priority by the tissues with a high turnover rate such as the intestinal tissues. It is noteworthy that the physico-chemical structure of the nitrogenous compounds present in the small intestine influences the intestinal uptake of arterial origin and its nature which is probably due to the changes it may cause in the kinetics of appearance of amino acids in the portal blood.

Glutamine metabolism in the enterocyte

The metabolism of glutamine in intestinal tissues has been widely studied during the last twenty years (Windmueller, 1982). This amino acid is supplied to the intestinal cell both via the arterial route and from the intestinal lumen. There is no doubt about the arterial pathway. Thus, after administration of protein free diets to pigs (Rerat et al., 1988c) glutamine represented 47% of the sum of amino acids taken up from the arterial blood by the gut wall. Windmueller (1980) considers that in most species and at each passage of blood in the intestine there is a net uptake of 20% à 30% of total plasma glutamine. More than 80% of glutamine infused into the mesenteric artery were immediately metabolised in the intestinal cells (Windmueller et Spaeth, 1974). This uptake of glutamine by the intestinal cell is as important as that of glucose, which is generally considered as the major source of energy in the gut (Krebs, 1972), but it depends on the blood concentration (Windmueller et Spaeth, 1974). Its utilisation seems to be lower in the ileum than in the jejunum (Hanson et Parsons, 1977).

Blood glutamine enters the enterocyte through the basolateral membrane by means of several transport systems (Mircheff et al., 1980), a sodium-independent, saturable and stereospecific system (L) and two sodium-dependent systems (A et ASC) ; these systems may transport a variety of neutral amino acids and are of high affinity for glutamine.

Glutamine may also originate from the intestinal lumen from which it is very rapidly absorbed, more rapidly than glutamate (Windmueller et Spaeth, 1975, 1976). It enters the enterocyte across the brush border by Na^+ dependent and Na^+ independent systems (Saïd et al., 1989). The fraction appearing in the portal blood increases with increasing luminal concentration. This is also the case for glutamate. In contrast, asparagine which is also easily absorbed is absolutely not metabolised in the rat gut cell because of the absence of asparaginase; this characteristic has not been found in the dog (Pinkus et Windmueller, 1977) or guinea pig (Friedhandler et Quastel, 1955).

The intestinal cell uses indifferently glutamine of both origins (Windmueller et Spaeth, 1975). The presence of glutamine in the intestinal lumen depressed the rate of utilisation of blood glutamine (20-40%) (Windmueller et Spaeth, 1975). In addition, in the presence of luminal glutamine its metabolism exceeded by 70% that of blood glutamine. The metabolism products of glutamine carbon chains are CO^2 (55%), lactate (8-14%), citrate (2-5%), citrulline (5%), proline (5%), alanine (4%) and other amino acids such as aspartate. These products have been evidenced *in vitro* (Windmueller et Spaeth, 1974) or *in vivo* (Windmueller et Spaeth, 1978). Their distribution being the same in conventional and axenic rats, they do not originate from the metabolism of the gut microflora. The major part of the carbon chain of alanine is derived from glucose (Windmueller et Spaeth, 1974) and lactate (Windmueller et Spaeth, 1978). Glutamine nitrogen was recovered quantitatively in a variety of substances in the portal blood, especially ammonia (38%), citrulline (28%) and alanine (24%), but also proline (7%) and some glutamate (2%) and ornithine (1%) (Windmueller, 1980)

The reactions at the origin of these substances are initiated by the presence of the corresponding enzymes. Thus, in most species the mucosa of the small intestine contains a phosphate dependent glutaminase (Katunuma et al., 1973). This activity was not modified by the presence of glutamine in the food, but it was reduced during a 48-72h fasting (Anderson et al., 1976). There is almost no glutaminase activity in the stomach and in the large intestine; the activity is located in the mitochondria of the villi and the epithelial crypts of the small intestine mucosa (Pinkus et Windmueller, 1977). It induces the deamination which is the initial reaction of a series leading to the formation of the above-mentioned substances. A very large fraction (80%) of amino nitrogen is transformed into ammonia and the rest into carbamoylphosphate which combined with ornithine leads to citrulline without any formation of urea or arginine (Windmueller et Spaeth, 1974).

Glutamate derived from the deamidation of glutamine can be reduced into proline or transaminated into ornithine ; by transamination with pyruvate a fraction of this glutamate may generate alanine and α-ketoglutarate. The enzymes catalysing these reactions are all present in the intestinal cell (Windmueller, 1982).

Which is the importance of glutamine metabolism in the enterocyte relative to its metabolism in the body ? In terms of nitrogen metabolism, glutamine generates various nitrogenous substances (ammonia, alanine, citrulline and proline) which are released into the portal blood and which may all constitute urea precursors (Lund et Watford, 1976). In pigs receiving a protein-free diet, the uptake of blood glutamine by the gastrointestinal tract represents about 5g/8h (Rerat et al., 1988c). If we assume that the substances produced from this glutamine are completely metabolised, the amount of urea synthesized within 8h may reach 2g (i.e. 6g/24h). The quantity of nitrogen bound to urea from glutamine (3g/24h) can thus be compared to current data of urinary nitrogen excretion obtained in pigs, i.e. 7 to 17g according to the dietary protein content (Rerat et Henry, 1964). It may be concluded that the proportion of glutamine nitrogen in this excretion is potentially high (18 à 43%). This confirms data obtained in fasting rats in which more than 30% of the nitrogen used in ureogenesis originate from the terminal products of intestinal glutamine metabolism (Windmueller et Spaeth, 1974). The ammonia proceeding from the metabolism of glutamine represents about 20% of the portal ammonia output in normally fed pigs (Rerat et Buraczewska, 1986 ; Rerat, 1988). In fasting dogs, this proportion ranges around 50% (Weber et Veach, 1979). On the other hand, the carbon chains of lactate, alanine et proline are available for gluconeogenesis (Ross et al., 1967). Besides, citrulline can be at the origin of arginine synthesis, mainly in the liver and kidney (Ratner, 1979). Arterial glutamine represents a preferential source of energy for the intestinal cell, its metabolism accounting for 38% of CO^2 produced versus 39% for aspartate and glutamate and only 6% for glucose (Windmueller et Spaeth, 1980). The role of glutaminolysis in the enterocytes and in other cells with a high division rate has been analysed (Newsholme et al., 1985).

Conclusions

A certain member of advances have been made during the last decade in our knowledge of protein digestion and absorption in monogastric animals.

These advances mainly concern :

- the enzymes involved in the terminal hydrolysis of proteins in the brush border and cytoplasm of the enterocytes, their variations according to the physiological status of the body and the analysis of their sequential action ;
- the transport systems of free amino acids and oligopeptides both in the brush border membrane and in the basolateral membrane of the enterocyte. These new data lead to interesting prospects concerning the use of mixtures of small peptides in human enteral nutrition ;
- the very active metabolism of the enterocyte both in terms of lipoprotein secretion to the blood and use of food and blood glutamine as metabolic "fuel" ;
- the hormonal regulations of some secretions such as the pancreatic secretions.These regulations contribute to explaining, until the molecular level, the adaptation of this secretion to the diet.

There are still many fields to be explored such as for instance :

- the adaptations of transport systems and their variations according to food and the physiological status of the body ;
- the hormonal regulations of intestinal secretions ;
- the possible appearance of native peptides in the portal blood.

This is not an exhaustive enumeration, but a survey of present knowledge and future prospects in the field of protein digestion and absorption.

Translated into English by Kirsten Rerat, INRA, Unité Centrale de Documentation, Centre de Recherches de Jouy-en-Josas

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THE DEVELOPMENT OF GASTRIC PROTEASES IN THE PRE- AND POST-NATAL PIG. THE EFFECTS OF AGE AND ACTH TREATMENT

P.T. Sangild[1], B. Foltmann[2] and P.D. Cranwell[3]

[1] Department of Animal Science and Animal Health, Royal Veterinary and Agricultural University, Rolighedsvej 23, 1958 Frederiksberg C, Denmark
[2] Institute of Biochemical Genetics University of Copenhagen, Øster Farimagsgade 2A, 1353 Copenhagen K, Denmark
[3] School of Agriculture, La Trobe University, Bundoora, Victoria 3083, Australia

Abstract

In this study we investigated the effects of age and treatment with adrenocorticotrophic hormone (ACTH) on the concentration of protease zymogens (prochymosin, pepsinogen and progastricsin) in stomach tissue and the pentagastrin-stimulated gastric secretion of protease activity (milk-clotting and general proteolytic activity). Fifty-five Large White x Landrace pigs from 22 days before birth (93 days gestation) to 36 days of age were used in the experiments. Prochymosin was present in fundic tissue 22 days before birth, reached peak concentrations at birth and decreased in concentration during the subsequent 36 days. In the first week after birth pepsinogen and progastricsin were absent or present in trace amounts but thereafter the concentrations of both zymogens increased rapidly. The age-related development of maximal pentagastrin-stimulated secretion of protease activity reflected the changes of zymogen concentrations in fundic tissue. Chronic treatment of pigs with ACTH from three days of age significantly increased the concentration of prochymosin in fundic tissue at 9-11 days and the concentrations of pepsinogen and progastricsin at 34-36 days of age.

Keywords: Gastric proteases; zymogens; chymosin; pepsin; gastricsin; development; pentagastrin-stimulation; ACTH treatment; cortisol

Introduction

The gastric juice of most mammals contains three groups of proteases: pepsin (EC 3.4.23.1), gastricsin (EC 3.4.23.3), and chymosin (EC 3.4.23.4). They are synthesized in the gastric mucosa as zymogens (pepsinogen, progastricsin and prochymosin) which are subsequently converted into active enzymes under acidic conditions. The gastric proteases are all homologous, but the individual groups show no immunochemical cross-reactivity (Foltmann, 1981). In adult pigs pepsin is the predominant gastric protease but minor amounts of gastricsin are also found. Chymosin has previously been regarded as characteristic for the gastric juice of young ruminants, but it is now recognized that chymosin occurs in the gastric juice of many newborn mammals including piglets (Foltmann et al. 1981). All the enzymes have milk-clotting activity, but the general proteolytic activity of pig chymosin is very small compared with that of pepsin or gastricsin. The aim of this work was to study the ontogeny of pig gastric proteases.

Earlier studies in young pigs have indicated that creep feeding and subsequent weaning on to solid food stimulated gastric secretory capacity of general proteolytic activity (Cranwell, 1985). Studies with rats have shown that development of gastric proteases can also be stimulated by hormones such as glucocorticoids (Ikezaki & Johnson, 1983; Yahav et al., 1986). We investigated protease zymogens in the stomach of young pigs after increasing plasma cortisol levels by chronic ACTH treatment. The effect on the pentagastrin-stimulated secretory capacity of milk-clotting and general proteolytic activity was also determined.

Materials and Methods

Fifty-five Large White x Landrace pigs, 0.4 to 13.5 kg body-weight from eight litters were used. The pigs of two litters were obtained by caesarean section, one litter at 93 days gestation (n=6) and the other at 105 days gestation (n=4). The remaining six sows farrowed normally and the piglets (n=45) were reared entirely by the sows; they had no access to solid food, no bedding was provided, and water was available ad lib. Nine of the piglets underwent gastric perfusion experiments at 0-1 or 5-7 days of age (Xu & Cranwell, 1990). The remaining 36 pigs were divided into 18 litter-mate pairs. One pig from each pair was injected intramuscularly with ACTH (12.5 $\mu g/kg^{0.75}$) twice daily from 3 days of age. The other pig of each pair (control) was injected with physiological saline. At 9-11, 16-18, 23-25 or 34-36 days of age littermate pairs underwent gastric perfusion experiments. Following a basal period, protease secretion was stimulated by intravenous infusion of pentagastrin during two consecutive 90 minute periods at dose rates of 4 and 8 $\mu g/kg/hour$ (Xu & Cranwell, 1990). After the perfusion experiments or following caesarean section each pig was euthanased with sodium pentobarbitone. The stomach was removed, emptied of any residual fluid, weighed and stored at -20°C until analysed. Procedures used in the preparation of the stomach tissue and gastric perfusate for analyses of enzymes, are described in detail by Sangild (1990).

The fundic region is the major site of synthesis of gastric protease zymogens in the pig (Linderstrøm-Lang et al., 1934); therefore samples of fundic tissue were used in this investigation. Zymogens in tissue extracts were characterized qualitatively by agar gel electrophoresis and detection of enzymes by milk clotting (Foltmann et al., 1985). Antisera were raised in rabbits against active enzymes and the concentrations of individual enzymes were determined by rocket immunoelectrophoresis after activation of the zymogens (Foltmann et al., 1981; Axelsson et al., 1983). Milk-clotting activity and general proteolytic activity in gastric perfusates were determined by radial diffusion techniques using bovine skim-milk and bovine haemoglobin as the substrates respectively (Lawrence & Sanderson, 1969; Samloff & Kleinman, 1969). Crystalline porcine pepsin (Sigma) was used as the standard in both assays. Units of activity were expressed as pepsin-equivalents; one unit represented the activity of 1.0 μg of pepsin.

Results

Each injection of ACTH in the treated pigs raised plasma cortisol levels 6-8 times above basal levels for 3-4 hours. Saline injection had no effect on plasma cortisol levels in control pigs. Treatment with ACTH had no significant effects on body weights or stomach weights at specific ages.

Prochymosin was present in the fundic mucosa 22 days before birth and reached peak concentrations (9 mg/g) immediately after birth (Figure 1). Although the tissue concentration of prochymosin decreased with age there was still more than 2 mg/g fundic tissue at 36 days. Pepsinogen could not be quantitated in fundic tissue until 5-7 days and progastricsin not until 9-11 days; concentrations of both zymogens increased with age with pepsinogen being predominant. Chronic administration of ACTH was generally associated with greater concentrations of each zymogen in fundic tissue. However, the differences between treated and control pigs were only significant at 9-11 days for prochymosin and 34-36 days for pepsinogen and progastricsin. The ratio of the tissue concentrations of pepsin to chymosin increased markedly from 9 to 36 days of age in both control pigs (0.1 to 2.8) and pigs treated with ACTH (0.1 to 5.9).

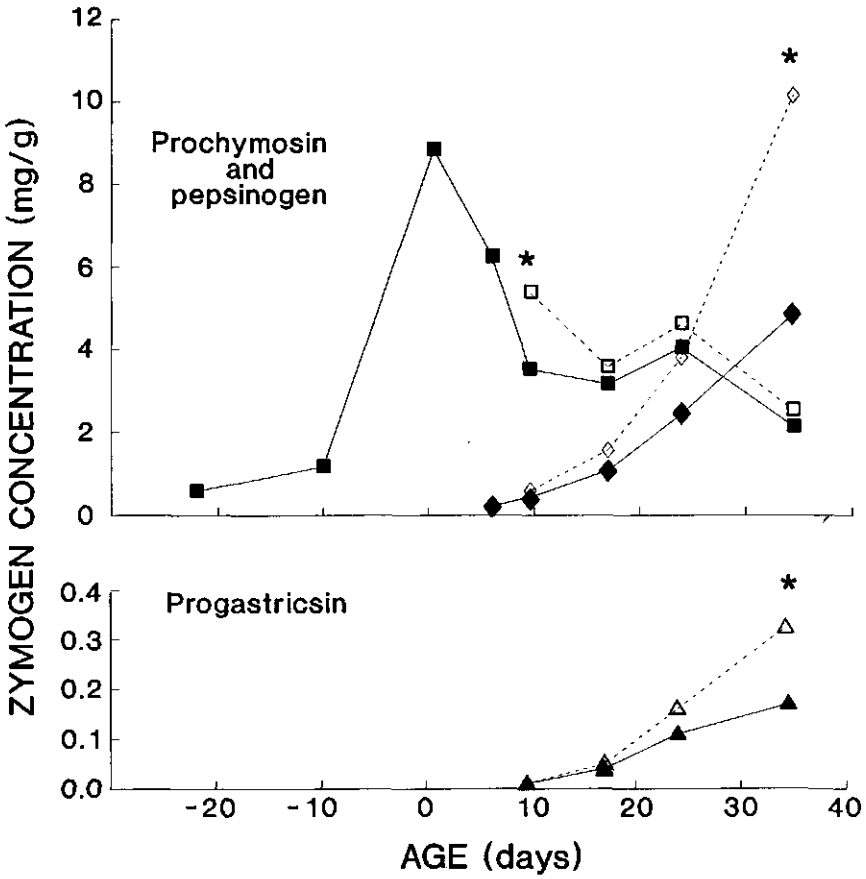

Figure 1. Concentration of zymogens (mean values) in fundic tissue from 22 days before birth to 36 days of age in control and ACTH-treated pigs. *: Differences between ACTH-treated and control pigs were significant ($P<0.05$). See text for details of pigs and treatments. Prochymosin: In control pigs (—■—) and ACTH-treated pigs (---□---). Pepsinogen: In control pigs (—◆—) and ACTH-treated pigs (---◇---). Progastricsin: In control pigs (—▲—) and ACTH-treated pigs (---△---).

The pentagastrin-stimulated secretion of milk-clotting and general proteolytic activities (Figure 2) showed a development which reflected concentrations of the relevant zymogens in fundic tissue (Figure 1). However, there were no significant differences between ACTH-treated and control pigs in the secretion of either activity.

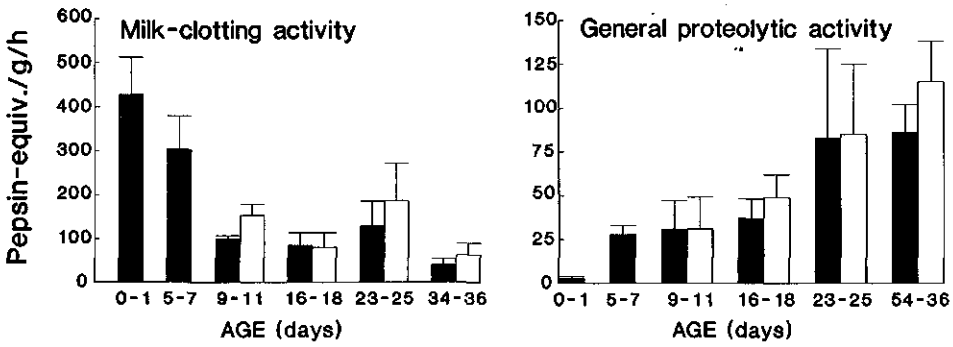

Figure 2. Maximal gastric outputs per hour of milk-clotting and general proteolytic activity per unit stomach weight in response to pentagastrin infusion at 4 and 8 μg/kg/h. See text for details of the pigs and treatments. Control pigs (■); ACTH-treated pigs (□).

Discussion

In the present study the highest tissue concentrations of prochymosin were found at or just around birth which agrees with the results of Foltmann et al. (1987). In the earlier study by Foltmann et al. (1981), the peak in tissue prochymosin concentration occurred during the 3 weeks before birth. The discrepancy between this observation and that in the present study could be due to inaccuracies associated with the methods of estimating fetal age by Foltmann et al. (1981) or to differences between pigs from different sources.

The physiological significance of chymosin being the predominant gastric protease in the first week of life is probably related to several factors. Whereas pepsin and gastricsin exert maximal general proteolytic activity at pH 2-3 chymosin is fully active at pH 3-5. During the first week after birth the acid secretory capacity is low (Sangild et al., 1989; Xu & Cranwell, 1990) and the synthesis of prochymosin may therefore ensure that the stomach of the pig has a high milk-clotting capacity in this period. The low general proteolytic activity of chymosin could be of importance for the newborn pig to prevent detrimental hydrolysis of immunoglobulins and other essential factors present in sow colostrum (Cranwell & Moughan, 1989). Furthermore, the casein clot allows the whey fraction of sows' milk, which includes the immunoglobulins, to pass rapidly to the small intestine (Noakes, 1971) where they are either taken up by enterocytes or act locally. Formation of a firm clot by chymosin would, in addition to regulating gastric emptying (Decuypere et al., 1986), also result in a degree of gastric distension which could be important to stimulate development of the stomach through physical effects or via an increased gastrin release (Stadaas & Schrumpf, 1974).

Apart from the studies of Foltmann et al. (1981, 1987) investigations into the development of gastric proteases in the young pig have relied on the measurement of general proteolytic activity in gastric mucosa, gastric contents and gastric secretions or measurements of the digestion of protein in the stomach (for a review see Cranwell & Moughan, 1989). Such studies have shown that the concentration of general proteolytic activity in gastric mucosa and gastric secretion, and the degree of digestion of protein in the stomach are low in pigs up to 3-4 weeks of age and then undergo a rapid increase. Thus, this reflects the transition from chymosin to pepsin as the predominant gastric protease during the first five weeks after birth (Figure 1).

Twice daily injections of ACTH stimulated the release of cortisol to levels which were within physiological limits although the responses may have been slightly higher than that produced by stressful conditions such as restricted feeding, cold exposure or chasing (Baldwin & Stephens, 1973; Rafai & Fodor, 1980). Treatment with ACTH stimulated the synthesis of the predominant zymogen of the gastric mucosa i.e. prochymosin in early life and pepsinogen and progastricsin in late development (Figure 1). Thus, the treatment seemed to increase the overall synthesis of gastric zymogens and did not affect the timing of the ontogenic changes from prochymosin to pepsinogen and progastricsin.

The secretory responses of milk-clotting activity and general proteolytic activity in the ACTH-treated pigs were often greater than those in control pigs but at no time were the age group means significantly different (Figure 2). There was a large variation between pigs within treatments and age groups, particularly at the two older ages. The extent of this variation was probably due, in part, to the nature of enzyme secretion which occurred in short bursts rather than continuously, as is the case for the pentagastrin-stimulated acid secretion (Sangild et al., 1989; Xu & Cranwell, 1990). The enzyme secretory capacity was therefore expressed as output per hour for the entire period of pentagastrin stimulation. However, it is also possible that pentagastrin did not produce maximal stimulation of either activities due to lack of gastrin receptors on the cells which produce the various zymogens involved. There is considerable variation between species in the occurrence of gastrin receptors on chief cells, i.e. the cells primarily responsible for the synthesis and secretion of pepsinogen. Distinct receptors for gastrin and CCK have been identified on chief cells from guinea pigs (Cherner et al., 1988) but gastrin receptors have not been found on chief cells from dogs (Soll et al., 1984). The authors are not aware of any published studies in which receptors to the various regulatory peptides and other secretagogues have been identified on porcine chief cells. As regards the ACTH-stimulated increase in zymogen synthesis and secretory capacity the present data correspond to those obtained with young rats injected with glucocorticoids (Ikezaki & Johnson, 1983; Yahav et al., 1986). However, the precocious maturation of chief cells reported in rat studies was not observed after treatment of young pigs with ACTH.

Earlier studies have indicated that stomach function in the young pig is influenced by age, intake of solid food and weaning (for reviews see Cranwell & Moughan, 1989; Sangild, 1990). In addition, the present results show that hormones such as ACTH and cortisol can stimulate the development of gastric proteases. The importance of such effects on gastric digestion in young pigs remains to be investigated.

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EFFECTS OF METHOD OF FEEDING AND MEAL SIZE ON THE POSTPRANDIAL GASTRIN RESPONSE IN PIGS

R-J. Xu*[1] , P.D. Cranwell* and D.P. Hennessy**

*School of Agriculture, La Trobe University, Bundoora, Victoria 3083, Australia
**Veterinary Research Institute, Attwood, 475-485 Mickleham Road, Attwood, Victoria 3049, Australia

Abstract

In Experiment 1 the effects of two different feeding methods (group v. individual) on the postprandial gastrin response were studied. Four groups of pigs (n = 4-6; 13 weeks - 3.5 years) were used, the animals were either housed and fed together or were housed and fed individually. In all pigs plasma gastrin concentration increased significantly within 15 minutes of commencement of feeding and remained elevated throughout the 3 hour period. Pigs housed and fed together had postprandial gastrin responses significantly greater than pigs housed and fed individually.

In Experiment 2 the effects of meal size on the postprandial gastrin response were studied. Pigs (n = 8; 12-13 weeks) were either fed a set meal of 500g or were allowed ad libitum access to feed for a period of 1 hour during which time their mean intake was 829 ± 127 g. Postprandial gastrin responses, similar to those observed in Experiment 1, were observed for all pigs irrespective of meal size. Maximal increases in plasma gastrin, and integrated postprandial gastrin responses, were significantly greater following a 1 hour feeding period than a 500g meal. Meal size has a significant positive effect on the postprandial gastrin response in the pig.

Keywords: Gastrin response; feeding method; meal size; young pig; sow

Introduction

Gastrin has dual effects on the gastrointestinal tract in that it stimulates the secretion of acid by the stomach and the proliferation of the mucosa in the stomach and small intestine (Walsh & Grossman, 1975; Johnson, 1987). In humans, following the introduction of amino acids or peptone solution into the stomach, gastric acid output has been shown to be highly correlated with an increase in serum gastrin concentration (Feldman et al. 1978; Lam et al. 1980). Also, increases in circulating gastrin concentration in response to feeding have been well documented (Walsh & Grossman, 1975), but little is known about the magnitude of the increase under different feeding regimes. In humans and pigs there is some evidence that meal size and the amount of protein ingested

[1] Present address: Department of Physiology and Anatomy, Massey University, Palmerston North, New Zealand.

influences the postprandial gastrin response (Blair et al. 1975; Woussen-Colle et al. 1977; Rerat et al. 1985). The aims of the present studies were to examine the effects of feeding pigs either individually or in groups, and varying the amount of food ingested on the postprandial plasma gastrin response.

Materials and Methods

Experiment 1

A total of 19 Large White x Landrace pigs were divided into four groups as follows: Group 1. 4 pregnant sows, 3.5 years old; Group 2. 6 pigs, 34 weeks old; Group 3. 4 pigs, 24.5 weeks old; and Group 4. 5 pigs, 13 weeks old. Pigs in Groups 1 and 3 were housed individually in pens measuring 1.5 m x 1.8 m, and pigs in Groups 2 and 4 were kept as two separate groups in two pens measuring 2.5 m x 3 m. Using procedures described by Xu (1989), each pig was prepared with a chronic venous catheter placed in a marginal ear vein or in a femoral vein one week prior to the experiment. Pigs were fed once daily with a commercial pelleted ration containing 15% crude protein (Barastoc, Victoria, Australia). The daily feed allowance was 4-5% of body-weight for the growing pigs, and 3 kg for the pregnant sows. After an overnight fast all pigs received their daily ration. Blood samples were taken at recorded intervals from 30 minutes before until 180 minutes after the start of feeding.

Experiment 2

Eight Large White x Landrace pigs, 10-13 weeks old and 17 to 26 kg body-weight, were housed in individual pens and fed once daily with a commercial ration containing 18% crude protein (Barastoc, Victoria, Australia) at 4-5% of body weight. Approximately one week prior to the experiments each pig was prepared with a chronic venous catheter placed in an external jugular vein according to the procedure described by Cranwell & Hansky (1980). Experiments were performed when the pigs were 12 and 13 weeks old. On two separate days in each week, and following an 18-22 h fasting period, the pigs were given either free access to food for one hour or a set meal of 500 g of food. Blood samples were taken at recorded intervals from 30 minutes before until 180 minutes after the start of feeding.

Plasma gastrin concentrations were measured by radioimmunoassay using antiserum PRN 1651 (Amersham, U.K.). The antiserum recognizes the mid-to-C-terminal region of G17 and has an equal cross-reactivity with both the G17 and G34 forms of human and porcine gastrin. The intra- and inter-assay coefficients of variation were 6.1-8.6% and 11.9-14.1% respectively. Statistical analyses were performed using paired and unpaired Students t-tests, and the least significant difference (LSD) test described by Steel & Torrie (1980).

Results

Experiment 1

Following an overnight fast, differences in basal plasma gastrin concentrations between the different groups of pigs were not significant (*Table 1*). In all pigs, the plasma gastrin concentration increased significantly ($P<0.05$) within 30 minutes after the start of feeding and remained elevated for the next 150 minutes (*Figure 1*).

Table 1. Basal concentrations, and maximal and integrated increases in plasma gastrin in individually-fed and group-fed pigs (Mean ± SEM).

Group No	n	Age	Feeding regime	Basal concentration (fmol/ml)	Maximal response# (fmol/ml)	Integrated response* (fmol/ml/min)
1	4	3.5 years	Individual	22 ± 2	16 ± 1[a]	9 ± 1[a]
2	6	34 weeks	Group	17 ± 1	29 ± 6[b]	19 ± 3[b]
3	4	24.5 weeks	Individual	20 ± 1	17 ± 4[a]	10 ± 1a
4	5	13 weeks	Group	17 ± 2	33 ± 6[b]	21 ± 2[a]

Peak concentration minus basal concentration.
* Mean integrated response of plasma gastrin above basal concentrations for a period of 3 hours after the start of feeding. Means within a column with different superscripts (a,b) were significantly different ($P < 0.05$).

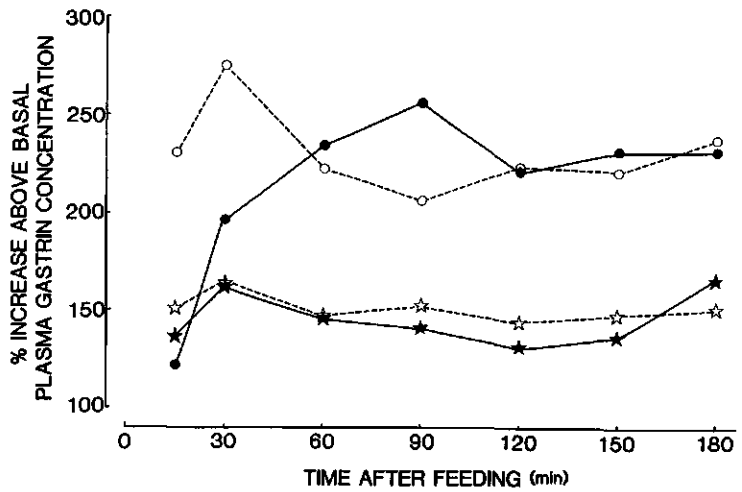

Figure 1. Mean percentage increase above basal plasma gastrin concentration in four 24.5-week-old pigs (☆) and four 3.5-year-old pregnant sows (★), which were housed and fed individually, and in five 13-week-old (o) and six 34 week old pigs (●), which were housed and fed in groups. The basal concentration of plasma gastrin was taken as 100%.

There was no apparent relationship between the age of animals and their postprandial increase in plasma gastrin concentration. However, there was an approximately two-fold difference in the postprandial gastrin response between individually-fed pigs and group-fed pigs. Similarly, the integrated gastrin responses were also significantly greater in the group-fed pigs than in the individually-fed pigs (*Table 1*).

Experiment 2

Feed intake during the one hour period in which pigs were given free access to food (*Table 2*) was positively correlated with body-weight at both 12 and 13 weeks ($P<0.05$; $P<0.01$ respectively).

Table 2. Meal size, and basal and postprandial increases in plasma gastrin concentration in eight pigs at 12 and 13 weeks of age (Mean ± SEM).

	12 Weeks		13 Weeks	
	Set meal	1h access	Set meal	1h access
Food consumed (g)	500	806 ± 144	500	850 ± 109
Gastrin:				
Basal (fmol/ml)	29 ± 1	30 ± 1	32 ± 1	30 ± 1
Maximal response† (fmol/ml)	11 + 2 *	18 ± 2	7 ± 2 **	22 ± 3
Integrated response‡ (fmol/ml/min)	5 ± 1 *	9 ± 1	2 ± 1 **	13 ± 3

† Peak concentration minus basal concentration. ‡ See *Table 1*.
Differences between treatments within weeks were significant (* $P<0.05$; ** $P<0.01$).

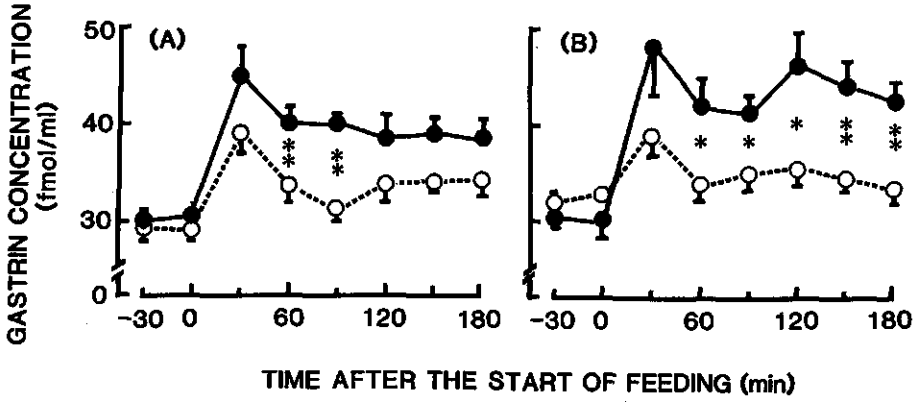

Figure 2. Plasma gastrin concentration before and after commencement of feeding in eight pigs at 12 weeks (A) and 13 weeks (B) of age (mean ± SEM). Following an overnight fast, the pigs were allowed either access to food for one hour (●) or a restricted meal of 500 g (o).

Ingestion of food following fasting evoked significant rises in plasma gastrin concentrations in all pigs on both feeding regimes (*Figure 2*). However, when pigs were given free access to food, the maximal and integrated gastrin responses were significantly greater than when they were given a 500 g meal (*Figure 2; Table 2*); the latter was usually consumed within a 30 minute period.

Discussion

Although competition for food may be a contributory factor in causing the greater postprandial gastrin response in group-fed pigs in Experiment 1, it is probable that the size of the meal eaten during the period of blood sampling was the major factor responsible for the difference between group- and individually-fed pigs. All pigs were given a similar amount of food per unit body-weight but it was observed that the group-fed pigs consumed their daily food allowance within a period of about one hour's duration, whereas the individually-fed pigs consumed their daily allowance in several meals over a period of eight hours.

A direct positive relationship between meal size and the postprandial gastrin response was demonstrated in Experiment 2 in which 12-13 week old pigs, restricted to a 500 g meal, had significantly lower maximal and integrated responses than when they were allowed ad libitum access to food for a 1 hour period and ate much larger quantities of food (829±127 g). Although no other evidence of the effect of meal size on the gastrin response in pigs could be found in the literature, similar results have been reported by Woussen-Colle et al. (1977) in humans in which the postprandial release of gastrin, measured as the integrated gastrin response, was positively related to meal size.

The precise effect that meal size has on the postprandial gastrin response was not determined in the present study. However, it could be related to the extent of gastric distension since Stadaas & Schrumpf (1974) and Stadaas et al. (1974) have reported that stepwise gastric distention causes progressive increases in serum gastrin concentrations in pigs. The effects of meal size on the postprandial gastrin response may also be related to the amount of certain nutrients ingested. Rerat et al. (1985) observed that a positive relationship existed between postprandial gastrin production and the amount of protein ingested by pigs. Another factor related to the magnitude of the postprandial gastrin response is the buffering capacity of ingested food. According to the results of recent studies by Bolduan et al. (1988) the commercial pelleted rations used in the present experiments would have considerable buffering capacity. Antral gastrin release is inhibited at low gastric antral pH (Walsh & Grossman, 1975). Thus, by increasing the amount of food eaten the quantity of acid which is buffered would be increased, the pH of gastric contents would remain relatively high and this in turn would prolong the stimulation of antral gastrin secretion.

The results of the present study have demonstrated that feeding regime, i.e. the pattern of food intake and the amount of food ingested during a meal, has a marked influence on gastrin secretion and thus on gastric digestive function. Further studies to determine more precisely how such factors as meal size, buffering capacity of the diet, type and amount of protein, and other

components of the diet influence gastrin secretion, subsequent gastric function and the action of other gut hormones and regulatory peptides should provide information useful for designing optimum feeding strategies for growing pigs.

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DOES PANCREATIC POLYPEPTIDE AFFECT GALLBLADDER FUNCTION IN THE PIG?

Annik LANGLOIS[1], Catherine JUSTE[1], Tristan CORRING[1], Catherine PHILIPPE[1], Jean-claude CUBER[2], Jean-Alain CHAYVIALLE[2]

[1] Laboratoire d'Ecologie et Physiologie du Système Digestif, I.N.R.A., Jouy-en-Josas, [2] INSERM, Hôpital E. Herriot, Lyon, France

Abstract

PP was shown to inhibit bile acid secretion in the pig. The present study aimed to determine if gallbladder function was affected by PP. Pigs were fitted with biliary and duodenal fistulae and a catheter was placed in a jugular vein for peptide infusion. Following a 8-day recovery period , infusion studies were performed after an overnight fast. Then, gallbladder was removed and experiments were repeated. PP did not affect basal biliary secretion either before or after cholecystectomy. On the contrary bile acid output stimulated by secretin and cholecystokinin was significantly inhibited by PP while bile flow rate was not affected. The magnitude of the decreases in bile acid output was in the same extent before and after cholecystectomy but in cholecystectomized pigs, inhibition of bile acid ouptut was delayed. It is concluded that PP did not affect mainly the gallbladder function.
Key words: biliary secretion, gastrointestinal peptide.

Introduction

The present study was undertaken to determine in the pig the effects of porcine pancreatic polypeptide (PP) upon basal and stimulated biliary secretion in the same animals before and after cholecystectomy.

Materials and Methods

Ten growing castrated male Large White pigs, weighing 40.6 ± 1.6 kg were fitted with biliary and duodenal fistulae. Bile was automatically restituted to the animals and continuously sampled for analysis on experimental days. Furthermore, a catheter was placed in a jugular vein for peptide infusion. Following a 8-day recovery period, infusion studies were performed after an overnight fast. Then gallbladder was removed and experiments were repeated in the same pigs.

In a first assay, we determined in 5 pigs the effect of a physiological dose of PP (LANGLOIS et al., 1990) on basal biliary flow and bile acid secretion. Isotonic saline solution containing 0.5% of porcine serum albumin was infused throughout the assay. 40 min after the start of infusion, 600 pmoles/kg/h of PP was infused for 60 min. Then NaCl infusion was continued for one hour more.

A second assay was performed to determine the effect of PP on stimulated biliary secretion in 8 pigs. Biliary secretion was stimulated by physiological doses of secretin (36 pmoles/kg/h) and of CCK-8 (600 pmoles/kg/h) (LANGLOIS et al., 1990) throughout the assay. 40 min

after the start of the infusion, 600 pmoles/kg/h of PP were infused for 60 min. Secretin plus CCK-8 infusion was continued for 1 h after PP infusion was stopped.

4% of the secreted bile were continuously removed and pooled as 10-min aliquots. In all bile samples, total bile acids were enzymatically determined.

Results and discussion

Before and after cholecystectomy, basal bile flow rate, bile acid concentration and output were quite similar and were not affected by intravenous PP infusion. In spite of a decrease in gallbladder pressure, ADRIAN et al (1982) found same results in bile flow during intravenous infusion of physiological doses of PP.

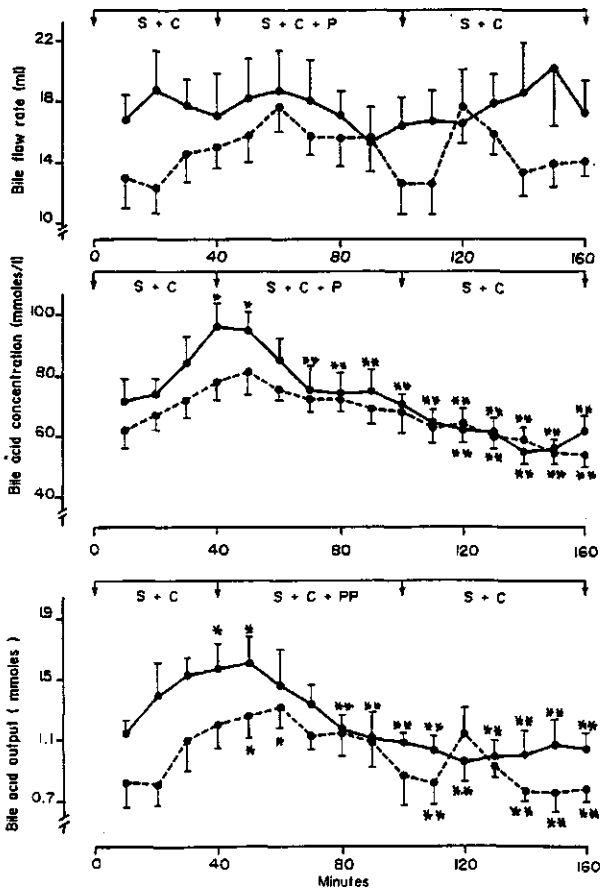

Fig.1. Kinetics of bile flow rate, bile acid concentration and bile acid output during intravenous infusion of 36 pmoles/kg/h of secretin and 600 pmoles/kg/h of CCK-8 (S+C) and during concomitant infusion of 600 pmoles/kg/h of porcine PP (S+C+P) in 8 pigs before (●———●) and after (●– – –●) cholecystectomy. * $P<0.05$ relative to the 0-10

min period of secretin plus CCK-8 infusion. ** $P<0.05$ relative to the 30-40 min period of secretin plus CCK-8 infusion before the start of PP infusion.

Secretin plus CCK-8 infusion induced significant rise in bile acid secretion both before and after cholecystectomy, but after gallbladder removal this increase was delayed (Fig.1). Before cholecystectomy PP infusion decreased bile acid concentration and output without affecting bile flow, and bile acid secretion remained dramatically reduced 60 min after the end of PP infusion (Fig.1) as previously shown (LANGLOIS et al., 1990). PP also inhibited bile acid concentration and output without affecting bile flow after cholecystectomy but these falls were retarded when compared to intact animals (Fig.1). But, cholecystectomy never caused any significant difference. At the end of the study, the magnitude of bile acid concentration and output decreases was in the same extent before and after gallbladder removal. So, PP does not appear to act only at gallbladder level in pigs. Until now, experiments were not conducted to determine the effect of PP on hepatic bile acid secretion or on intestinal bile acid absorption. However, specific PP receptors are present on basolateral membrane of the canine small intestine suggesting that PP participates in the regulation of nutrient absorption (GILBERT et al., 1988). Thus, it will be interesting to evaluate the effect of intravenous PP infusion on intestinal absorption of bile acid.

In conclusion, PP had no effect on basal biliary secretion but significantly inhibited stimulated bile acid secretion without affecting bile flow rate, both before and after cholecystectomy. It can be suggested that PP actively participates to postprandial regulation of biliary secretion in the pig and that it does not act mainly at the level of gallbladder. In fact, gallbladder has a minor role in bile delivery into the duodenum in this animal species (data not shown).

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PIG ILEAL DIGESTIBILITY ASSOCIATED WITH ANTINUTRITIONAL FACTORS AND PROTEIN QUALITY IN PHASEOLUS BEANS AND SOYABEANS

A.F.B. van der Poel[1], B. Kemp[1], E.R. Wahle[1] and D.J. van Zuilichem[2]

[1] Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands.
[2] Department of Food Technology, section Process Engeneering, Bomenweg 2, 6703 HD Wageningen, The Netherlands

Abstract

Feeding of Phaseolus beans, steam processed at high temperature (HT; ~136 °C) for a short time (ST; 1.5 min) to piglets caused a large increase in the ileal digestibility of nitrogen. The positive effect of this HTST treatment is not likely to be attributed to differences in the residual levels of lectin proteins and trypsin inhibitors present in heat processed beans. These factors were virtually eliminated with the used processing conditions. The increase in in vivo digestibility, therefore, may be attributed to qualitative changes in the properties of the bean storage proteins after heating. In contrast to Phaseolus beans, the ileal digestibility of protein from soyabeans was shown to be less temperature dependant. The processing time at a certain temperature obviously is an important variable to control an optimal ileal digestibility of soyabean protein.

The effects of the different processing methods on the utilization of lysine and other amino acids from heated beans in metabolism, remain to be investigated.

Introduction

Animal feed manufacturing involves the use of a variety of raw materials to produce complete diets. These diets are defined according to nutritional and technological specifications to meet the requirements of several animal species. Some raw materials, for example certain legume seeds, can satisfy these specifications only after some form of processing.
The genus Phaseolus vulgaris includes all species of legume seeds normally known as common beans. The protein nutritional value of most bean-type designations of these beans (Kidney, Great Northern, Navy etc.) is limited in a number of aspects compared with animal proteins (Sgarbieri & Whitaker, 1982). This is also the case for the protein nutritional value of soyabeans (Glycine max) The presence of toxic proteins and other so-called antinutritional factors (ANF) may influence the protein utilization of these beans. These factors are mainly associated with lectins, protease inhibitors and in some varieties also with polyphenols. In additions to ANF, the Phaseolus bean protein itself is somewhat resistant to enzymatic attack (Liener and Thompson, 1980; Van der Poel, 1990) and/or antinutritional properties (Santoro et al., 1989).

The upgrading of beans by processing is largely based on thermal treatments which are effective to decrease the level of lectins (Antunes & Sgarbieri, 1980) and the activity of protease inhibitors (Rackis et al., 1986). The possible change in susceptibility of the bean proteins itself to proteolysis after heat treatment and the subsequent impact on ileal in vivo protein digestion, however, has not yet been clarified (Van der Poel, 1990).
The results presented here estimate the magnitude of different steam treatment procedures for whole Phaseolus beans and soyabeans. Analytical aspects of bean proteins and ANF from Phaseolus have been reported previously (Van der Poel et al., 1990). The effects of inclusion of steam treated beans in diets for piglets on the ileal digestibility of protein were evaluated.

Materials and Methods

Steam processing was carried out using a laboratory-scale pressurized toaster provided with an inlet and a discharge sluice as described by Van der Poel & Van Zuilichem (1991). This type of equipment enables high temperature/short term (HTST) processing in addition to low temperature/ long term (LTLT) treatments. After steaming to the desired pressure (temperature), batches of beans were metered in the inlet sluice. This sluice was operated at a feed rate providing a single layer of beans on the belt conveyor. The batch was held for different residence times under specific heating conditions with maximum temperature tolerances of 0.5°C being attained. Beans were processed in a randomized order. After steam processing all samples were immediately air-dried (35°C for 24 hours) prior to milling (stepwise: 6 and 1 mm, resp.), storage (4°C).

Two in vivo experiments were carried out with castrated male piglets fitted with a post-valvular T-caecum (PVTC) cannula (Van Leeuwen et al., 1988). Ileal digestibility was determined using 5 animals per treatment with an average weight of 21.5 ± 1.2 kg (Phaseolus trial) and 30.0 ± 1.7 kg (soyabean trial).

The experiments comprised a 100% control diet as well as experimental diets in which 20% of the control diet was replaced by beans, steam processed at different conditions of intensity. The control diet was formulated to supply dietary protein by casein and herring meal. The Phaseolus beans and soyabeans used in the experiment were steam heated with processing conditions shown in Table 1.

Table 1. Processing temperature and duration (min) for steam treatments[1]

	Pressure (temperature)								
	100 kPa(102 °C)					300 kPa(119 °C)			400 kPa[2]
	10	20	40	60	80	2	5	7	1.5
Phaseolus beans		*	*	*	*		*		*
Soyabeans	*	*	*			*		*	*

[1] Laboratory-scale toaster [2](135°C)

All diets were pelleted without steam addition and were supplied at a feeding level of 2.6 times the energy requirement for maintenance.
The animals were accustomed to the experimental diets for 11 days. Ileal chyme was collected in plastic pouches 12 h a day and for 5 days following the adaptation period. Apparent ileal digestibility coefficients were calculated with reference to chromic oxide (Cr_2O_3) as the indigestible marker.

Results

The average values for apparent digestibility of Phaseolus beans are presented in Figure 1.

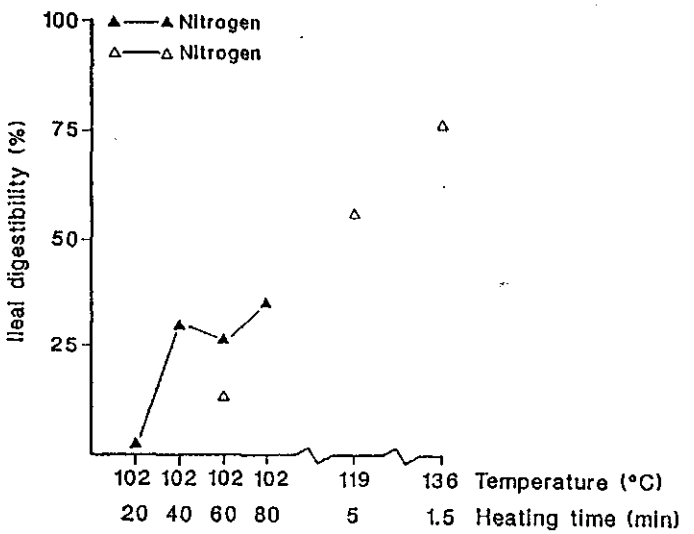

Figure 1. Apparent ileal digestibility of protein from steam processed common beans (Van der Poel et al., 1991; Livest. Prod. Sci., In Press)

The apparent ileal digestibility coefficients of dry matter and nitrogen from Phaseolus beans showed large differences between the diets at 20% inclusion level. Steaming of beans showed consistent differences ($P<0.05$) for the digestibility values with respect to the temperature/time pattern of heating. Heating at 102°C or at 119°C did affect the diet ileal digestibility of dry matter and nitrogen significantly ($P<0.05$) as compared to the control diet.

For soyabeans data on the apparent ileal digestibility of dry matter, nitrogen and ether extract for piglets in relation with processing conditions are given in Figure 2.

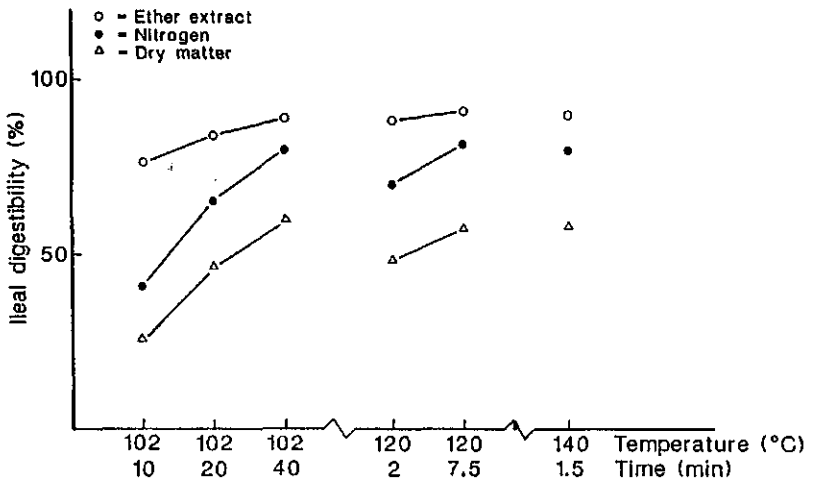

Figure 2. Apparent ileal digestibility of dry matter, nitrogen and ether extract from steam processed soyabeans

The steaming of whole soyabeans at 102 °C and 120 °C for longer processing times clearly increases the apparent digestibility of nutrients under investigation. Obviously, the highest ileal digestibility values obtained for dry matter, nitrogen and ether extract are similar within each temperature treatment. Thus, no clear advantage was observed for HTST processing in view of in vivo digestibility.

Discussion

A comparison was made of beans, heat processed at different temperatures and processing times. The heat processed beans had previously shown to inactivate lectins (ELISA) and TIA to a similar low level (Van der Poel et al., 1990). Processing times, while ensuring more or less complete destruction of heat sensitive lectins and TIA may result in nutritional damage (Bender, 1984). It was shown that short processing e.g. at 119°C, needed to inactivate ~95% of lectins resulted in a relatively small decrease in in vitro lysine availability.

HTST steaming procedures of beans did restore ileal digestibility of nitrogen and lysine in beans diets to the level of the control group. These results lead to the conclusion that the low level of both lectins and TIA in beans, after steam processing at different conditions, cannot be a significant cause for the relatively large differences in ileal digestibility found for these beans. Presumably, changes in the storage protein (Glycoprotein II; Phaseoline) with HTST processing contribute to higher digestibility values. This effect is not observed for steaming at 102°C which can be explained by the presumably higher temperature needed for denaturation of the storage protein at the relatively low moisture levels used at steaming.

For soyabeans, however, the results show that the inactivation of ANF (particularly TIA) is rather complete under different temperature/time relationships of steam heating, all of them which to ensure similar values for ileal digestibility for dry matter, protein and ether extract

in piglets of 30 kg of age. In the case that different temperature/time regimes establish similarity for bean quality in relation with ileal digestibility values, HTST treatments are in favour in view of higher throughputs of the steam processing equipment.

In conclusion it is suggested to employ HTST-procedures rather than LTLT-procedures for steaming of both Phaseolus beans and soyabeans, based on the kinetics for ANF inactivation. The former conditions will increase the in vivo protein digestibility of Phaseolus beans as determined in pig experiments. The effect of processing on the bioavailability of amino acids, lysine in particular, requires further investigation.

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STIMULATORY EFFECT OF DIETARY CHANGES AT WEANING ON THE EXOCRINE PANCREAS IN DEVELOPING PIGS

B.R. Weström, S.G. Pierzynowski, J. Svendsen & B.W. Karlsson

Department of Animal Physiology, University of Lund, Helgonavägen 3B, S-223 62 Lund, Sweden.

Abstract

To study the relationship between the time for abrupt weaning and the induction of maturation of the pancreas function, Swedish Landrace pigs were surgically prepared with chronic catheters permitting sampling of pancreatic juice and blood on conscious animals during their postnatal development up to and after weaning.

The data obtained show that the increase in pancreas secretion during postnatal development is independent of age at weaning (4 or 6 weeks) but rather appears to be related to the dietary change from sow milk (the piglets were not creep-fed) to dry solid food. Other factors, e.g. loss of regulating factors in milk and "stress" factors released at weaning, may influence the changes in pancreas function.
Key words: Exocrine pancreas, amylase, trypsin, lipase, postnatal development, weaning.

Introduction

In the postnatal pig marked increases in the enzyme contents of pancreatic homogenates and in intestinal contents have been reported to occur, as well as qualitative changes in enzymatic composition (Corring et al., 1978; Owsley et al., 1986; Weström et al., 1987). The observations were recently extended in studies on pure pancreatic juice obtained from piglets in acute experiments (Harada et al., 1988) and from pigs with chronic pancreas duct catheters (Pierzynowski et al., 1990). In the latter study marked quantitative and qualitative changes in the exocrine pancreas function was noted after 4-5 weeks of age, i.e., at the time for weaning. Moreover, the ingestion of sow milk had little effect on the pancreas secretion, while ingestion of solid food after weaning had a marked post-prandial stimulating effect. Since the study was performed under standard managemental conditions and the piglets had access to creep fed from the 2nd week of life it was impossible to determine if the observed developmental changes in pancreatic function was due to age or dietary changes.

The present investigation was undertaken to study the effect of abrupt weaning, at either 4 or 6 weeks of age, on the development of the exocrine pancreas in pigs only nursed by their sow up to weaning.

Materials and Methods

Eight purebred Swedish Landrace piglets were fitted at an age of 14-15 days with pancreatic catheters and duodenal fistulae for periodic external sampling of pancreatic juice, and a jugular vein catheter for blood sampling according to the method described by Pierzynowski et al. (1988). In each of 2 litters 4 pigs were operated and returned to their sow, usually within 12 h, together with 4 unoperated littermates. The

piglets were nursed up to weaning at either 4 or 6 weeks and no creep feeding was given. After weaning, the pigs were housed in separate cages with visual contact with each other, and were offered a dry weaning food ad lib (Växfor, Lantmännen, Stockholm, Sweden).

Beginning 2 days after surgery, pancreatic juice was collected twice a week before and after food ingestion. Starting for the nursing pigs 1 h after removal from the sow and for the weaned pigs after overnight fasting, 'basal' secretion was collected during 4 x 15 min periods. The pigs were then fed during 30 min; the nursing pigs obtained sow milk by sucking their sows and the weaned pigs were given a standard meal of 10 g Växfor/kg b.wt. Then an additional 4 x 15 min postprandial collections were made. The volume of the pancreatic juice from each collection was determined and samples were stored at -20 °C and analysed.

Analysis

The pancreatic juice samples were analysed for total protein content using a micro-adaptation of the Lowry method. Trypsin (EC.4.4.21.4) activity was determined after enterokinase activation using Na-benzoyl-DL-arginine-p-nitroanilide as substrate, where one unit (U) was defined as the amount of enzyme which hydrolyses 1 µmol substrate per min (Pierzynowski et al., 1990). Amylase (EC.3.2.1.1) activity was analysed according to Ceska & Birath (1969) using the Phadebas Amylase reagent (Pharmacia, Uppsala, Sweden). Lipase (EC.3.1.1.3) activity was determined by a pH-stat titration method using tributyrin as substrate (Erlanson-Albertsson et al., 1987) and expressed as µmoles fatty acids released per min and ml.

Results

As shown in Table 1, the basal pancreatic juice outflow as well as the output of total protein and lipase were constant during the whole experimental period, while the basal output of amylase and trypsin activity slightly increased after weaning.

For the nursing piglets (preweaned), no response to feeding was obtained, since the postprandial levels were equal to the basal levels for all parameters studied. However, after weaning in piglets weaned at either 4 or 6 weeks, a significant increase in all parameters were obtained after feeding solid food.

Discussion

The data obtained in the present study using chronically catheterised pigs confirmed previous observations and showed, in addition, that abrupt weaning can trigger maturation of the secretory capacity of the pancreas, independently of weaning at 4 or 6 weeks of age.

Some differences in the development of the individual enzymes could be noted, since an increased basal secretion after weaning was observed only for trypsin and amylase, while the basal levels remained unchanged for lipase. In addition, the trypsin/protein and amylase/protein ratios increased in the pancreatic juice after weaning, whereas the lipase/protein ratio was stable during the whole period studied. The observed general increase in enzyme secretion and the proportional increase of trypsin and amylase production indicated that there was both an increase in the number of acinar cells in the pancreas per kg body weight with development and a specialisation of the cells with a higher gene expression for some enzymes with age. These changes of the

Table 1. Levels (mean ±SD, n = 8) of pancreatic juice outflow (ml/kg/h), output of total protein (mg/kg/h), trypsin, amylase and lipase (U/kg/h), during 1 h before feeding (basal) and 1 h after feeding sow milk before weaning or solid food after weaning (post-prandial) in chronically catheterized pigs.

A: weaning at 4 w.

Age		3-4 w (preweaned)	5-6 w (weaned)	7-8 w (weaned)
Volume	basal	1.3 ± 0.6	1.4 ± 0.5	1.4 ± 0.1
	postpr.	1.0 ± 0.5^{a}	3.2 ± 1.7^{b*}	3.5 ± 1.0^{b*}
Protein	basal	3.9 ± 3.7	3.4 ± 1.7	5.4 ± 2.4
	postpr.	3.8 ± 2.3^{a}	12.7 ± 8.4^{b*}	31.0 ± 6.2^{c*}
Trypsin	basal	0.6 ± 0.6^{a}	1.5 ± 1.4^{b}	2.9 ± 1.6^{b}
	postpr.	0.7 ± 0.5^{a}	5.4 ± 4.1^{b*}	18.1 ± 5.5^{c*}
Amylase	basal	90 ± 110^{a}	250 ± 250^{b}	340 ± 320^{b}
	postpr.	60 ± 40^{a}	670 ± 560^{b*}	2300 ± 1800^{c*}
Lipase	basal	280 ± 120	480 ± 370	380 ± 340
	postpr.	320 ± 170^{a}	1100 ± 670^{b*}	2800 ± 2300^{b*}

B: weaning at 6 w.

Age		3-4 w (preweaned)	5-6 w (preweaned)	7-8 w (weaned)
Volume	basal	0.9 ± 0.5	1.2 ± 0.7	1.4 ± 0.7
	postpr.	1.0 ± 0.7^{a}	0.9 ± 0.6^{a}	2.9 ± 1.8^{b*}
Protein	basal	3.1 ± 2.8	2.7 ± 2.0	4.4 ± 3.6
	postpr.	3.5 ± 3.2^{a}	3.3 ± 2.7^{a}	14.1 ± 7.1^{b*}
Trypsin	basal	0.5 ± 0.6^{a}	0.7 ± 0.6^{a}	2.0 ± 2.0^{b}
	postpr.	0.6 ± 0.7^{a}	0.8 ± 0.7^{a}	5.8 ± 4.1^{b*}
Amylase	basal	50 ± 70^{a}	40 ± 30^{a}	390 ± 230^{b}
	postpr.	50 ± 70^{a}	50 ± 50^{a}	1100 ± 530^{b*}
Lipase	basal	320 ± 330	400 ± 330	700 ± 440
	postpr.	340 ± 320^{a}	580 ± 670^{a}	2100 ± 1500^{b*}

Statistically significant differences were evaluated between basal and postprandial values (vertical) using paired Student's t-test, where *$p < 0.05$, and between age groups (horizontal) with Student's t-test, where different letter superscripts indicate differences at the $p < 0.05$ levels between the parameters.

pancreatic function during postnatal development can be explained as adaptation of the pancreas to a diet of solid food containing 2-3 times more protein and carbohydrates than fat as compared to sow milk.

The increased pancreas function in the piglet appears to be independent of age (4 or 6 weeks), but rather to be dependent on the dietary change from sow milk to dry solid food at weaning. Several possibilities for the mechanisms of induction of pancreas maturation at weaning can be suggested. Firstly, changes in gastro-intestinal hormonal release as a response to the dietary change might function as triggering factors. Results obtained by Korc et al. (1981) and Wicker et al. (1984) support this, since food components were shown to selectively stimulate the production of hormones affecting transcriptional processes in the acinar cells, e.g., a high carbohydrate diet increases the insulin levels that influence the pancreatic amylase. Secondly, the presence of milk components or milk digestive products in the intestines may delay the maturation; their disappearance at weaning could be considered as the factor initiating the development of the exocrine pancreas. Thirdly, abrupt weaning with a surge of "stress" hormones could play a role as initiating factors. Glucocorticoids provoked maturation of exocrine pancreas function as well as other GI tract functions in both the rat (Henning, 1987) and in the pig (Chapple et al. 1989). However, preliminary results on blood serum cortisol levels in the developing piglet showed that they decreased at weaning, a fact which would argue against the latter hypothesis.

In conclusion, our results suggest that the development of the pancreas function in pigs depended on the dietary change from sow milk to dry solid food at weaning rather than on age, since weaning at either 4 or 6 weeks of age gave the same results. More generally, these developmental changes appear to be related to profound dietary changes, since it has been shown that nursing piglets reach pancreatic maturation concomitant with the start of ingestion of significant amounts of creep feed (Corring et al., 1978).

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EFFECTS OF PEA ANF'S AND PEA CARBOHYDRATES ON ILEAL PROTEIN DIGESTIBILITY OF PIGLETS

J. Huisman[1] and M.P Le Guen[2]

1) TNO-Institute of Animal Nutrition and Physiology (IGMB-Dept. ILOB), PO Box 15, 6700 AA Wageningen, The Netherlands.
2) EURETEC GIE, 12, Avenue George V, 75008 Paris, France.

Abstract

A study was made to investigate whether the low apparent ileal protein digestibility of raw peas is related to antinutritional factors (ANFs; mainly trypsin inhibitors and lectins) or to carbohydrates or to both fractions. To study this the following preparations were made from a spring and from a winter pea variety (FINALE and FRIJAUNE, respectively):

1. pea protein isolate from which the ANFs and carbohydrates were removed;
2. pea ANF-concentrate containing high levels of trypsin inhibitors and lectins;
3. fraction consisting exclusively of pea carbohydrates.

Apparent ileal digestibility of these three fractions was measured in experiments with young piglets. In the first experiment pea carbohydrates were included in diets differing in protein sources: casein + fish or protein isolate from FINALE peas or protein isolate from FRIJAUNE peas. There was no reduction in apparent ileal protein digestibility due to the addition of pea carbohydrates. The small intestinal chyme flow, however, was increased when pea carbohydrates were included in the diets.

In the second experiment, ANF concentrate (consisting mainly of trypsin inhibitors and lectins) was included in a diet with pea protein isolate from FINALE. A significant reduction in apparent ileal protein digestibility and weight gain was found.

Introduction

Reduction of performance in piglets fed a high level of Pisum sativum in diets has been reported by various authors (Castaing and Grosjean, 1985; Fekete et al., 1984; Bengala-Freire et al., 1989; Grosjean and Castaing, 1983; Grosjean et al., 1986; Grosjean and Gatel, 1986, 1989). In previous studies it was shown that the apparent ileal protein digestibility of pea protein isolate (from which ANFs and the carbohydrates were removed) was distinctly higher than in raw peas. The aim of the present investigation was to study whether ANFs or carbohydrates are responsible for these marked differences in ileal protein digestibility. Therefore, the following fractions were prepared: pea protein fraction containing low levels of ANFs and no carbohydrates, a pea ANF-concentrate containing high levels of trypsin inhibitors and lectins, and a fraction consisting of a mixture of soluble and insoluble pea carbohydrates. These fractions were used in experiments with piglets to study which factor is responsible for the differences in ileal protein digestibility between raw pea and pea protein isolate.

Two ileal digestibility experiments with piglets were carried out. In the first experiment pea carbohydrates were included in diets differing in protein sources. The aim was to investigate whether pea carbohydrates do affect ileal protein digestibility and to test whether there is an interaction between effects of the carbohydrates and the protein source. In the second experiment, an ANF-concentrate was added to a diet with pea protein isolate from FINALE as the protein source. The objective was to test whether pea ANFs affect the apparent ileal protein digestibility.

Materials and Methods

a Preparation of pea protein isolate, ANF concentrate and the carbohydrate fraction.

The pea protein isolate was prepared at INRA-Nantes (France) according to Guéguen (1983). The carbohydrate concentrates consisted of a mixture of the insoluble carbohydrates fraction produced during the isolate process and the soluble carbohydrates prepared by 50 °GL aqueous ethanol extraction (temperature 70 °C) of the raw pea four in the ratio 3/1 v/w, the mixture being then spray-dried. The quantities of the two components of the mixture were chosen to give about the same proportions of insoluble/soluble carbohydrates as in raw peas.

Table 1. Chemical composition (%) of the pea protein isolates and ANF-concentrate.

Nutrient	FINALE	FRIJAUNE	ANF CONCENTRATE 4)
Dry matter	96.5	95.9	96.0
Ash	2.3	2.8	4.0
Crude protein	89.5	88.3	61.9
Crude fat	8.0	8.0	n.d.
TIA 1)	0.6	1.6	49.1
Lectins 2)	1394	1604	101944
Tannins 3)	<0.1	<0.1	<0.1

1) TIA: mg inhibited trypsin per g product measured according to Van Oort et al. (1989)
2) ELISA µg/ g product
3) % catechins, measured with the vanillin sulphuric acid method according to Kuhla and Ebemeier (1981).
4) Mix of ANF concentrates from the varieties FINALE and FRIJAUNE
n.d.= not determined.

Table 2. Chemical composition (%) of the pea carbohydrate (insoluble and soluble) fractions.

	FINALE	FRIJAUNE
Dry matter	96.6	96.8
Crude ash	1.8	1.3
Crude protein	4.6	2.5
Crude fibre	8.0	11.4
Saccharose	2.5	1.0
Raffinose	0.2	0.1
Stachyose	1.2	0.8
Lectins 1)	6.3	8.5
TIA 2)	0.2	0.3
Tannins 3)	<0.1	<0.1

1) ELISA: µg lectins/ g product
2) TIA: mg inhibited trypsin per g product
3) % catechins, measured with the vanillin sulphuric acid method.

b Diets and treatment groups.

In **experiment** 1, the following experimental groups were formed:

I : protein source: casein and fish; carbohydrate source: mix of cornstarch + dextrose
II : protein source: casein and fish; carbohydrate source: mix of cornstarch + dextrose + pea carbohydrates
III: protein source: pea protein isolate FINALE; carbohydrate source: mix of cornstarch + dextrose
IV : protein source: pea protein isolate FINALE; carbohydrate source: mix of cornstarch + dextrose + pea carbohydrates
V : protein source: pea protein isolate FRIJAUNE; carbohydrate source: mix of cornstarch + dextrose
VI : protein source: pea protein isolate FRIJAUNE; carbohydrate source: mix of cornstarch + dextrose + pea carbohydrates.

The carbohydrate composition of test diets II, IV and VI was made up as close as possible to the carbohydrate composition in the raw pea diets used in previous studies.

In **experiment** 2, two diets were formulated; a control diet with low levels of trypsin inhibitors and lectins, and a test diet with high levels of these ANFs. The protein source in both diets was pea protein isolate from FINALE, which had a low ANF activity. A high level of ANFs in the test diet was obtained by the inclusion of ANF concentrate. The carbohydrate sources in the diets were corn maize starch and dextrose. The diets in both experiments were balanced for contents of protein, amino acids, energy and minerals.

The diets of both experiments were pelleted without steam at about 50°C. The pellet size was 3 mm.

c Animals and experimental procedure.

The piglets in both experiments were of the crossbred Dutch Landrace x Dutch Yorkshire. They arrived at the Institute at an age of 4 weeks. Their mean live weight on arrival was approximately 8 kg in both experiments. After an adaptation period of 6 days, the piglets were surgically fitted with a post-valve T cannula (PVTC) according to van Leeuwen et al. (1988). After surgery, the piglets were allowed to recover from surgery and adapt to the experimental diets for 19 days. Following this period, the ileal chyme was collected on 8 consecutive days, 12 hours per day, in experiment 1, and during 5 consecutive days, also 12 hours per day, in experiment 2. The collection period in experiment 2 was shorter than that of experiment 1, because the amount of ANF concentrate available was limited. The chyme was collected in plastic bags attached to the cannula, and weighed every hour before being immediately stored in a freezer (-20 °C). Before analysis, the total collected chyme was pooled per animal, homogenized and sampled. The age of the piglets during the ileal chyme collection period, was about 8 to 9 weeks. Their mean live weight was about 15 kg.
In both experiments, the piglets were fed restrictedly at 2.6 times their maintenance energy requirement. Feed was administered twice daily. Water was freely available from nipple drinkers.

d Statistical analysis.

The results are given as means with their standard deviations (Tables 3 and 4). Analysis of variance were carried out according to procedures described by Steel and Torrie (1960). The differences between treatments were analysed using the Student's t-test. The values in Table 3 were analysed according to a two-way analysis of variance according to Snedecor and Cochran (1967).

Results and Discussion

Inclusion of pea carbohydrates in the diets did not affect the apparent ileal protein digestibility (Table 3). Dry matter digestibility of the test diets with pea carbohydrates was significantly lower ($P<.05$) compared with the control diets containing no pea carbohydrates. This effect was observed with each protein source (Table 3). The results of the dry matter digestibility indicate that the ileal digestibility of the pea carbohydrates is lower than that of cornstarch + dextrose. The dry matter content of ileal chyme of the piglets fed the pea carbohydrates, was lower ($P<.05$) compared with the piglets fed the diets containing no pea carbohydrates (Table 3). The amounts of ileal chyme, excreted by the piglets fed the three diets containing pea carbohydrates, was higher ($P<.05$) than those fed the control diets without pea carbohydrates. The increased chyme flow may be related to the fact that the pea carbohydrates were incompletely digested in the small intestine.

Table 3. Mean dry matter contents in ileal chyme and apparent ileal digestibility of dry matter and crude protein of the diets of experiment 1.

	Protein source	Number of animals	Carbohydrate source	Dry matter content ileal chyme		Ileal digestibility Crude protein		Dry matter	
				VALUES PER TREATMENT					
				MEAN	SD	MEAN	SD	MEAN	SD
I,	C + F	4	C + D	13.56a	1.19	87.1a	2.1	86.1a	1.2
II,	C + F	4	C + D + Pea	11.10b	1.19	84.8a	2.3	82.7bcde	1.0
III,	FI Is	4	C + D	12.05ab	0.43	88.2a	2.4	84.1ce	1.1
IV,	FI Is	3	C + D + Pea	10.86b	0.53	86.7a	1.7	80.8d	1.8
V,	FR Is	3	C + D	14.07a	1.95	85.8a	1.9	84.5ae	1.1
VI,	FR Is	3	C + D + Pea	13.23b	1.82	86.0a	1.9	81.9bcd	0.3
				EFFECT OF CARBOHYDRATE SOURCE; COMPOSITE DATA.					
				MEAN	SEM	MEAN	SEM	MEAN	SEM
Diets I, III, and V.				13.15x	0.38	87.12x	0.63	84.93x	0.35
Diets II, IV, and VI				11.66y	0.40	85.73x	0.66	81.86y	0.37

Protein source: C + F = Casein + Fish; FI Is = FINALE protein isolate; FR Is = FRIJAUNE pea protein isolate.
Carbohydrate source: C + D = Corn starch + Dextrose.
a-e: means in the same column that do not have a common letter, differ significantly ($P<0.05$).
x-y: means in the same column that do not have a common letter, differ significantly ($P<0.02$).

Table 4. Apparent ileal N digestibility, and live weight gain measured in experiment 2.

Diet	Number of animals	Ileal N digestibility		Live weight gain	
		mean	SD	g/day	SD
PPI diet	5	86.0a	2.3	277a	29
PPI diet + ANF concentrate	5	78.9b	2.8	229b	25

PPI= Pea protein isolate.
Means with superscripts that do not have a common letter in the same column, differ significantly ($P<0.05$)

As a result, more osmotic active components will be present in the chyme. The osmotically active carbohydrates in the chyme will cause an inflow of water into the intestinal lumen in order to maintain osmotic equilibrium between blood and lumen content. The results of Table 4 clearly demonstrate, that with the addition of the pea ANF-concentrate containing high levels of trypsin inhibitors and lectins to diets, the ileal protein digestibility is reduced. As could be expected with a lower ileal protein digestibility, the daily weight gain in the piglets was significantly lower (17%) with the diet enriched with ANFs (Table 4). Summarizing the results, it can be concluded that ANFs are related to the low apparent ileal protein digestibility with raw peas and pea carbohydrates are not.

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SIMULTANEOUS HEPATIC AND GUT BALANCES OF AMINO ACIDS IN THE PIG

C. SIMOES NUNES, I. GALIBOIS*, A.RERAT and L. SAVOIE*

Département NASA, INRA-CRJ, 78352 Jouy-en-Josas Cedex, France and
* Département NHC, Université Laval, Québec, G1K7P4 Canada

Abstract

In vivo hepatic and intestinal balances of amino acids, insulin and glucagon were studied in six pigs after ingestion of casein (13.9% - CA 12 or 27.8% - CA 24) or rapeseed proteins (23.2% - RA 12). Amino acids (aa) from all diets were very well absorbed. Hepatic uptake of total aa (taa) in 12 h expressed as a percentage of absorbed quantities was 13% for CA 12, 66% for CA 24 and 25% for RA 12. Differences in the hepatic extraction rate of essential aa (eaa) appeared between the two levels of casein ingestion and for ARG between the two protein sources. The liver showed always a net production of ASP and GLU. The production and hepatic balance of insulin were the lowest after ingestion of RA 12 whilst no differences were noted for glucagon. Independently of the nutritional situation, the hepatic extraction rate of insulin appeared to be higher than that of glucagon.
Keywords: Amino acids, liver, balances, insulin, pig.

Introduction

In the pig there are numerous experimental data on the fluxes of nutrients and other substrates in the portal-drained viscera (Rérat et al., 1984; 1985). However, the hepatic fluxes have only considered some substrates (Simoes Nunes et al., 1984; 1989). Estimation of aa fluxes in the splanchnic area has been made in the dog (Elwyn et al., 1968; 1972; Barrett et al., 1986) whilst in other species including man (Wahren et al., 1976) the data are either very fragmentary or inexistant. The aim of the present work was to evaluate the entering and leaving liver fluxes of aa in the pig, the role of the liver in their metabolisation as well as in that of two pancreatic hormones - insulin and glucagon - after ingestion of two very different proteins, casein and rapeseed concentrate.

Materials and Methods

Six pigs (64±4.8kg) were fitted with three catheters, placed in the portal vein, brachiocephalic artery and right hepatic vein, respectively as well as with two electromagnetic flow probes, one around the portal vein and the other around the hepatic artery as described by Simoes Nunes et al. (1989). After a preliminary adaptation to each diet, the animals received at a one-week intervals and according to a double latin square design, three test meals of 800 g each, one containing 23.2% rapeseed concentrate (diet RA 12) and the others 13.9 or 27.8% hydrochloric casein (diets CA 12 and CA 24)(table 1). Each observation period lasted for 12 h. Portal vein and hepatic artery blood flow-rates were recorded continuously and blood was sampled fourteen times simultaneously from the three vessels for aa and hormone determinations.

Table 1. Composition of experimental diets (%) and level of amino acid (aa) intake (mg) from each test meal.

	CA 12	CA 24	RA 12
Hydrochloric casein (UCCP)	13.9	27.8	-
Rapeseed concentrate (CETIOM)	-	-	23.2
Peanut oil	6	3	3
Maize starch	65.5	54.6	62.7
Purified cellulose	6	6	2.5
Mica powder	5	5	5
Mineral mixture	2.5	2.5	2.5
Vitamin mixture	1	1	1
Antioxidant	0.1	0.1	0.1
∝ essential aa	51792	100872	48496
∝ non essential aa	58408	113968	56352
∝ total aa	110200	214840	104848

Results

The absorbed quantities of taa after ingestion of diet CA 24 were about twofold higher than those absorbed after ingestion of the other two diets (hourly means: 18241±1222mg-CA 24, 9048±1188mg-CA 12 and 9057±1324mg-RA 12). After 12 h, these quantities represented for all diets approximately the sum of ingested individual aa. The quantities of individual eaa absorbed after ingestion of CA 24 were significantly larger than those absorbed after CA 12 and RA 12 except for MET. There was no significant difference between the quantities of eaa absorbed after ingestion of diets CA 12 and RA 12. GLN showed a negative absorption after CA 12 and RA 12 and slightly positive after CA 24. The absorption percentages of ASP and GLU appeared to be very low. Opposite to that, those of ALA and GLY appeared to be very high. The quantity of aa entering the liver after ingestion of diet CA 24 was approximately 10% higher than that measured after diet CA 12. The latter represented 120% of the flux of aa entering the liver in animals fed diet RA 12. Whatever the diet, the eaa accounted for about 40% of the mixture of

Table 2. Hourly means (mg) of the quantities of amino acids (aa) taken up by the liver and percentage (%) of absorbed aa taken up by the liver in 12 h in the pig after ingestion of diets CA 12, CA 24 and RA 12.

	CA 12	CA 24	RA 12
Essential aa mg/h	$1591\pm562^{(1)a}$	5814 ± 842^{b}	1689 ± 365^{a}
Total aa mg/h	1149 ± 745^{a}	12072 ± 2057^{b}	2240 ± 624^{a}
Essential aa %	$36\pm13^{(2)a}$	67 ± 10^{b}	40 ± 9^{a}
Total aa %	13 ± 8^{a}	66 ± 11^{b}	25 ± 7^{a}

(1) Mean ± standard deviation of the mean of 6 determinations.
(2) Mean ± standard deviation of the mean of 6 determinations expressed as a percentage of the absorbed quantity.
a.b. Significantly different at the level of 1 %.

taa entering the liver. The quantities of eaa and taa taken up by the liver after ingestion of CA 24 were significantly higher than those taken up after CA 12 and RA 12. This was true in terms of real amounts of aa as well as in percentage of the absorbed quantities of aa (table 2). Within the mixture of aa taken up by the liver, the eaa represented 138, 48, and 75%, respectively of the mixture of taa for CA 12, CA 24 and RA 12. Among the eaa, the quantities of VAL, THR, PHE, LYS and HIS retained by the liver were significantly higher after intake of CA 24 than after CA 12 and RA 12 (table 3). There was a negative hepatic

Table 3. Hourly means (mg) of the quantities of some amino acids taken up by the liver (HB) and mean hepatic extraction coefficients (HEC) in the pig after ingestion of diets CA 12, CA 24 and RA 12.

		CA 12	CA 24	RA 12
VAL	HB (mg)	$48\pm173^{(1)a}$	927 ± 410^{b}	-22 ± 54^{a}
	HEC	1 ± 2^{a}	9 ± 4^{b}	-0.2 ± 0.7^{a}
PHE		290 ± 109^{a}	839 ± 111^{b}	255 ± 90^{a}
		11 ± 4^{a}	23 ± 3^{b}	12 ± 3^{a}
LYS		239 ± 206^{a}	1181 ± 255^{b}	310 ± 122^{a}
		2 ± 2^{a}	11 ± 2^{b}	4 ± 2^{a}
MET		107 ± 77	127 ± 92	57 ± 39
		7 ± 6	6 ± 4	4 ± 3
ARG		104 ± 85^{a}	462 ± 141^{b}	324 ± 92^{b}
		3 ± 3^{a}	13 ± 4^{b}	9 ± 2^{ab}
ASP		-384 ± 108	-259 ± 178	-258 ± 112
		-30 ± 8	-21 ± 14	-25 ± 11
GLU		-2374 ± 432^{a}	-1125 ± 399^{b}	-1810 ± 461^{ab}
		-37 ± 7	-23 ± 8	-31 ± 7
ALA		890 ± 181^{a}	1450 ± 183^{b}	648 ± 319^{a}
		15 ± 3^{a}	31 ± 4^{b}	12 ± 6^{a}

(1) Mean ± standard deviation of the mean of 6 determinations.
a.b. Significantly different at the level of 5 %.

uptake (liver output exceeding liver input) of VAL after ingestion of RA 12. The quantity of ARG retained by the hepatic parenchyma after diet RA 12 was significantly higher than after CA 12. Among the eaa, PHE showed the lowest non hepatic tissue uptake (32 and 25%) and VAL the highest (92 and 104%) after ingestion of CA 12 and RA 12 respectively. In the case of CA 24, the two aa showing the extreme values for non hepatic tissue uptake were PHE (11%) and MET (72%). The quantity of aa leaving the liver after intake of CA 24 was only 3% higher than that measured after CA 12. This small difference was due to a proportionally higher hepatic aa uptake after ingestion of CA 24 than after that of CA 12. The hepatic output of aa after intake of CA 12 accounted for 119% of that observed in animals fed diet RA 12. Inside the aa mixture leaving the liver, the eaa accounted for 36% of the sum of aa after ingestion of CA 12 and RA 12; after that of CA 24, they

accounted for 41%. The hepatic uptake of non eaa (neaa) after intake of CA 24 feeding was generally higher than that measured for the other two diets (table 3). Nevertheless, the individual differences were not significant for ASP, GLY, PRO, P-SER, TAU and ORN. The hepatic balances of ASP and GLU were negative after all the three diets, but the hepatic production of GLU was much smaller after ingestion of CA 24 than after that of the other two diets. The lowest mean production of insulin was observed after ingestion of diet RA 12 (table 4). This production represented only 72% of that noted after ingestion of the two casein diets. The hepatic extraction coefficients of the produced insulin and of the hormone exposed to the liver appeared to be very similar for all the three diets (table 4). No significant difference between the three diets appeared concerning the production, kinetic profile of production, liver input fluxe and hepatic extraction coefficient of glucagon.

Table 4. Hourly means of productions (P), hepatic balances (HB) of insulin (mU/h) and glucagon (ng/h) and mean hepatic extraction coefficients according to the produced quantity of hormone (HEP) or according to the quantity of hormone exposed to the liver (HEC) in the pig after the ingestion of diets CA 12, CA 24 and RA 12.

		CA 12	CA 24	RA 12
Insulin	P	2735±270(1)a	2723±230a	1972±220b
	HB	1656±200a	1569±190a	1146±150b
	HEP	54±10	58±9	58±3
	HEC	21±7	25±5	24±2
Glucagon	P	24938±5875	28800±5712	26870±5493
	HB	12849±3867	11566±6468	10715±3660
	HEP	52±5	40±1	40±8
	HEC	11±1	8±3	9±2

(1) Mean ± standard deviation of the mean of 6 determinations.
a.b. Significantly different at the level of 5%.

Discussion

Blood plasma is the main interorgan vehicle of most aa. Nevertheless, some aa such as GLU, GLN and TAU are found in higher concentrations in the red cells than in blood plasma and thus can participate in intertissue aa transfers (Elwyn et al.,1972). This was the reason why we used the measurement of free aa in the whole blood. A good aa absorption balance was observed after ingestion of the casein based diets. That finding was largely expected since casein is a good quality protein. More surprising was the absorption balance of taa measured after rapeseed concentrate feeding which appeared particularly high for a plant protein especially if considereing what was observed with cereal proteins (Rérat et al.,1979). The absorption percentages noted twelve hours after the test meals suggest an optimum uptake and transport of oligopeptides and aa resulting from the intraluminal digestive hydrolysis of dietary and endogenous proteins as well as from those resulting from the metabolism of the gut wall. The absorption percentages were higher than those observed by Galibois et al. (1989)

eight hours after intake of similar diets. Interesting differences between the two sources of proteins concerned the balances of individual neaa appearing in the portal vein. The low absorption coefficients of ASP and GLU as well as the high absorption coefficients of ALA and GLY were the result of deamination and transamination reactions in the intestinal wall during the absorptive process (Windmueller and Spaeth, 1980). The intestinal balance of GLN was either slightly positive or negative indicating a large metabolic utilization of this aa by the intestinal wall. CIT and ORN were synthetized by the intestinal wall. CIT seemed to originate from metabolisation of GLN and ORN from that of dietary and blood ARG (Windmueller and Spaeth, 1980).

Whatever the diet ingested by the animals, PHE, ALA, and SER showed the highest hepatic uptake coefficient. Among the branched chain aa, VAL showed the lowest hepatic uptake and the highest non hepatic tissue uptake. A low hepatic uptake of branched chain aa was also observed in the dog suggesting a large participation of these aa in nitrogen transfers from the liver to the non hepatic tissues (Elwyn, 1970; Bloomgarden et al., 1981; Barrett et al., 1986).

It was of considerable interest that the hepatic extraction level of MET was not modified by the quantity of ingested protein. Hence the quantity of MET reaching and utilized by the non hepatic tissues was larger after ingestion of CA 24 than after that of CA 12.

The very high hepatic productions of ASP and GLU (Gelfand et al., 1986) in the three different nutritional situations must be underlined. It seems that hepatic behaviour of ASP and GLU in the pig is different of that of the dog in which during the post-prandial period there is a hepatic extraction of both diacids.

Two of the glucose forming aa, ALA and SER, showed the highest level of hepatic extraction. This level appeared also to be highly increased by the ingestion of a high casein diet. Both aa are carbon sources for glucose synthesis (Kaplan and Pitot, 1970) and the NH_2 radicals of ALA participate in the observed high hepatic productions of GLU.

The differences noted in the hepatic extraction level according to the quantity and nature of ingested proteins underline that the liver profoundly modifies the profile of the aa mixtures entering the organ. The "buffer" role of the liver in the availability of dietary and endogenous aa for the non hepatic tissues can no more be considered as "uniform", but as being rather selective.

The lowest mean hourly production of insulin was observed after ingestion of rapeseed proteins. The lower glucose absorption after intake of this diet (Simoes Nunes et al., 1989) was very likely the main reason for the difference observed between casein and rapeseed diets.

Independently of the nutritional situation, the level of hepatic extraction of circulating insulin was proportionally much higher than that of glucagon. In the dog, the hepatic extraction coefficient of insulin was also much higher than that of glucagon (Röjdmark et al., 1978; Ishida et al., 1983).

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DEVELOPMENT OF INTESTINAL DISACCHARIDASES, INTESTINAL PEPTIDASES AND PANCREATIC PROTEASES IN SUCKING PIGS. THE EFFECTS OF AGE AND ACTH TREATMENT

P.T. Sangild[1], P.D. Cranwell[2], H. Sørensen[3], K. Mortensen[3], O. Norén[4], L. Wetteberg[4] & H. Sjöström[4]

[1] Department of Animal Science and Animal Health, Royal Veterinary and Agricultural University, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark
[2] School of Agriculture, La Trobe University, Bundoora, Victoria 3083, Australia
[3] Chemistry Department, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.
[4] Department of Biochemistry C, The Panum Institute, University of Copenhagen, Blegdamsvej 3c, DK-2200 Copenhagen N, Denmark

Abstract

Three disaccharidase activities (lactase, sucrase, maltase) and three peptidase activities (aminopeptidase N, dipeptidyl peptidase IV, aminopeptidase A) were measured in mucosal extracts of the proximal small intestine from sucking pigs. The activities of two proteases (trypsin and chymotrypsin) were measured in extracts of pancreatic tissue. Forty-one Large White x Landrace pigs from birth to 5 weeks of age were used in the experiments; littermate pairs were treated with either physiological saline or adrenocorticotrophic hormone (ACTH) from 3 days after birth. Sucrase and maltase activities were low in newborn pigs but increased with age. At 5 weeks the ACTH-treated pigs had significantly higher activities than the control pigs. The activities of lactase and the three peptidases were well developed at birth, decreased during the postnatal period and were not affected by ACTH treatment. Trypsin and chymotrypsin activities increased from birth to 5 weeks of age. Pigs treated with ACTH tended to have higher trypsin activities at 5 weeks than control pigs. The results indicate that hormones such as ACTH and glucocorticoids may influence the postnatal development of sucrase, maltase and trypsin activities in the young pig.

Keywords: Small intestine; pancreas; disaccharidases; peptidases; proteases; development; ACTH treatment; cortisol

Introduction

The mucosa of the small intestine and the pancreatic glands produce a number of hydrolases which play an important role in the final digestion of nutrients (Norén et al., 1986; Alpers, 1987). The postnatal development of the different hydrolases varies among species but in general it reflects the changes in dietary intake from milk during the suckling period to solid food after weaning. Intestinal lactase activity is high in newborn pigs and decreases after birth (Hartmann et al., 1961; Manners & Stevens, 1972). Sucrase, maltase, trypsin and chymotrypsin activities are low at birth, but they increase during the postnatal period (Hartmann et al., 1961; Corring et al., 1978; Weström et al., 1987). Intracellular peptidases has been found in the intestinal mucosa of both fetal and sucking pigs (Lindberg & Karlsson, 1970). There are no reports on the development of brush border peptidases in the pig.

Development of digestive enzymes may be influenced by factors such as the external environment, feed intake, genetics and hormonal regulation. Treatment of young rats with glucocorticoids is known to induce early maturation of some intestinal and pancreatic enzymes (for a review see Henning, 1981). In the present

study we investigated the effect of ACTH treatment on some enzyme activities in the small intestine of sucking pigs: lactase (ß-D-galactosidase, EC 3.2.1.23), sucrase (sucrose α-D-glucohydrolase, EC 3.2.1.48), maltase (isomaltase & maltase-glucoamylase; oligo-1,6-glucosidase & α-D-glucosidase, EC 3.2.1.10 & EC 3.2.1.20), aminopeptidase N (microsomal aminopeptidase, EC 3.4.11.2), dipeptidyl peptidase IV (EC 3.4.14.5) and aminopeptidase A (aspartate aminopeptidase, EC 3.4.11.7). Furthermore, trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) activities were measured in pancreatic tissues.

Materials and Methods

Forty-one Large White x Landrace pigs from 7 litters were used in the experiments. The pigs were reared entirely by the sow; they had no access to solid food, no bedding was provided and water was available ad lib. Eleven pigs were used to study enzyme development from birth to seven days of age; the remaining 30 pigs were divided into littermate pairs and used to study the effects of age and ACTH treatment from 9 to 36 days. One pig from each pair was injected intramuscularly with ACTH (12.5 $\mu g/kg^{0.75}$) twice daily from 3 days of age. The other pig of each pair (control) was injected with physiological saline. Littermate pairs were killed at 9-11, 16-18, 23-25 or 34-36 days of age with an overdose of sodium pentobarbitone. Two newborn pigs were fasted for only 8 hours but the remaining pigs were fasted for approximately 24 hours before they were killed. The fasting period was necessary to prepare the pigs for studies on gastric function (Sangild et al., 1989). The abdomen was opened, the pancreas removed, and a 10 cm segment of the small intestine excised 20 to 30 cm from the pyloro-duodenal opening (proximal jejunum). The intestinal segment was opened lengthwise and gently rinsed in cold physiological saline. The tissues were stored at -20°C until analysed.

After thawing, mucosa of the intestinal segment was scraped off the underlying muscular layers, homogenized and sonicated in aqueous 1% Triton X-100. After centrifugation this procedure was repeated with the sediment and the two supernatants were combined and used for analysis. Lactase, sucrase, maltase, aminopeptidase N, dipeptidyl peptidase IV and aminopeptidase A activities were determined using the methods described by Sjöström et al. (1978) and Sørensen et al. (1982). The respective substrates used in the assays were lactose, sucrose, maltose, L-alanine-4-nitroanilide, glycyl-L-proline-4-nitro-anilide and L-α-glutamic acid 4-nitroanilide. Protein concentration was determined in the mucosal homogenate by the method of Lowry et al. (1951). Intestinal enzyme activities were expressed as units per mg of mucosal protein. One unit was equal to 1 μmol substrate hydrolyzed per minute.

While frozen, pancreatic tissue was cut into fine pieces and proenzymes were extracted in a Tris-HCl buffer (pH 8.0). After centrifugation and activation of proenzymes (Elnif et al., 1988) enzyme activities were measured in the supernatant (Geiger & Fritz, 1984). The substrates used to detect trypsin and chymotrypsin activities were N-α-benzoyl-L-arginin-4-nitroanilid-hydrochloride and glutaryl-L-phenylalanin-4-nitroanilid. The activities were expressed as units per mg of tissue and one unit was equal to 1 μmol substrate hydrolyzed per minute.

Results

In the treated pigs each injection of ACTH raised plasma cortisol levels 6-8 times above basal levels for 3-4 hours. Such cortisol levels are comparable to those following stressful conditions such as restricted feeding and chasing (Baldwin

& Stephens, 1973; Rafai & Fodor, 1980). Saline injection had no effect on plasma cortisol in the control pigs. Treatment with ACTH had no significant effects on body weight or stomach weight at specific ages.

Intestinal enzyme activities are shown in Figure 1. After the first postnatal week sucrase and maltase activities increased significantly with age ($P<0.05$) and at 34-36 days the activities were significantly higher in ACTH treated pigs than in control pigs ($P<0.05$). Lactase and peptidase activities decreased with age and there were no significant effects of ACTH treatment. At 34-36 days of age these enzyme activities were significantly lower ($P<0.05$) than the average activities during the first week after birth.

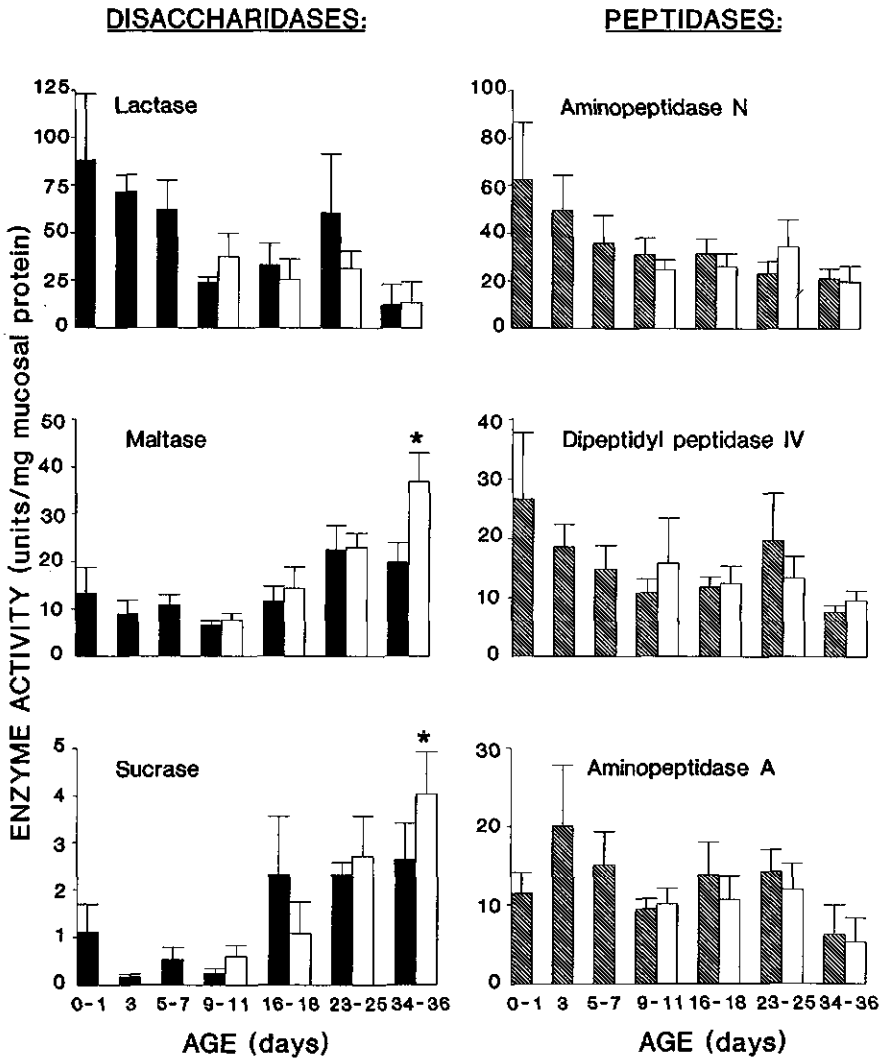

Figure 1. Development of disaccharidase activities (lactase, maltase, sucrase) and peptidase activities (aminopeptidase N, dipeptidyl peptidase IV, aminopeptidase A) in the small intestine of sucking pigs. (Mean ± SEM, n=3-4). (■; ▨) control pigs; (□) ACTH-treated pigs.

Protease activities measured in extracts of pancreatic tissues are shown in Figure 2. The specific activity of chymotrypsin increased significantly with age ($P<0.05$). Age had no consistent effect on the activity of trypsin and a temporary decrease in trypsin activity was observed at 23-25 days ($P<0.05$). The ACTH-treated pigs tended to have higher trypsin activities than the control pigs at 34-36 days but no significant differences between the two treatment groups were detected.

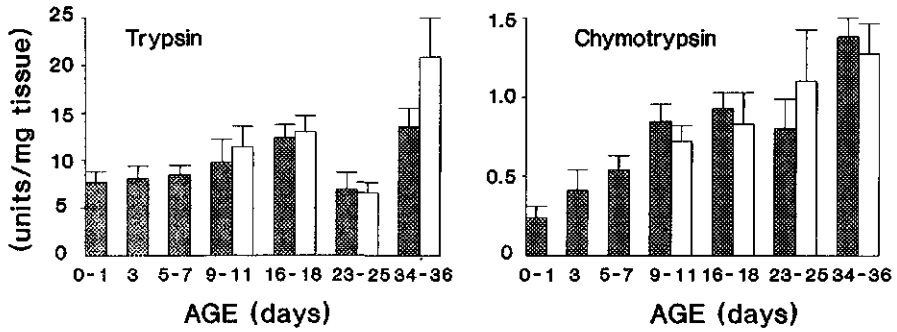

Figure 2. Development of pancreatic trypsin and chymotrypsin activities in sucking pigs. (Mean ± SEM, n=3-4). (■) control pigs; (□) ACTH-treated pigs.

Discussion

The observed intestinal disaccharidase and peptidase activities showed a large variation among pigs of the same age and treatment which is consistent with findings in earlier investigations (Lindberg & Karlsson, 1970; Manners & Stevens, 1972; Corring et al., 1978; Kidder & Manners, 1980; Chapple et al., 1989). The distribution of each enzyme along the intestine is also known to vary with age and among species. In general, lactase activity is most abundant in the proximal small intestine and absent in the distal ileum. Most other enzymes are found along the entire length of the intestine with maximal activities in the proximal and middle regions (Kidder & Manners, 1980; Norén et al., 1986; Alpers, 1987). In the present investigation, enzyme activity measurements were restricted to the proximal small intestine and the observed developmental pattern can therefore be used only as a rough guide to what occurs along the entire intestine.

The decrease in lactase activity and increases in maltase and sucrase activities (Figure 1) are in agreement with the findings in earlier studies (Hartmann et al., 1961; Manners & Stevens, 1972; Corring et al., 1978; Chapple et al., 1989). The parallel postnatal increase of sucrase and maltase activities is expected because the enzyme complex sucrase-isomaltase (EC 3.2.1.48 and EC 3.2.1.10) is responsible for all intestinal sucrase activity and a large part of the maltase activity (Sørensen et al., 1982; Norén et al., 1986). The remaining maltase activity mainly originates from the enzyme maltase-glucoamylase (EC 3.2.1.20).

The changes in disaccharidase activities can be accelerated by weaning and by feeding diets rich in sugar and starch (Manners & Stevens, 1972; Kidder & Manners, 1980). Specific enzymatic changes also take place in fetal and sucking animals but without any obvious "substrate induction". Enzymatic development is therefore controlled not only by diet but possibly also by genetic and hormonal factors. Treatment of sucking pigs with ACTH or glucocorticoids had no consistent effects on the development of sucrase and maltase activities in the recent study of Chapple et al. (1989). However, the observed stimulatory effects of ACTH on maltase and sucrase activities at 5 weeks of age in the present study (Figure 1)

indicate a potential regulatory role of adrenal hormones in the postnatal development of such disaccharidase activities. Discrepancies with the findings of Chapple et al. (1989) could be explained by differences in sources of animals, treatments, sampling methods and tissue analysis.

The activities of the three brush border peptidases appeared to decrease with age although the trend was less pronounced for aminopeptidase A than for the other enzymes (Figure 1). This postnatal development corresponds to that of the intracellular dipeptidases. These enzymes increase in activity during late gestation with peak values occurring in newborn pigs (Lindberg & Karlsson, 1970). During the fetal period peptidases may have a physiological role to ensure intestinal hydrolysis of the various small peptides and amines in amniotic fluid swallowed by the fetus. An early fetal development of peptidase and lactase activities may also explain why ACTH treatment did not influence these enzyme activities after birth.

The activities of pancreatic proteases increased during the first 5 weeks of age but this trend was more clear for chymotrypsin than for trypsin (Figure 2). The temporary decrease in trypsin activity at 23-25 days was unexpected and there could be a connection to the slightly increased lactase and peptidase activities (Figure 1) and increased gastric chymosin activity (Sangild et al., 1989) at this age. Other investigators have reported similar postnatal developments of trypsin and chymotrypsin activities (Hartmann et al., 1961; Corring et al., 1978; Weström et al., 1987). These studies have shown that major increases in pancreatic protease activities also occur in the fetal period and after 5 weeks of age.

Treatment with ACTH had no effect on chymotrypsin activity but ACTH tended to stimulate trypsin activity at 34-36 days of age. At this age the ACTH-treated pigs also had higher concentrations of cathionic trypsinogen in plasma than the control pigs (53±6 versus 27±6 ng/ml) (Sangild & Weström, unpublished results). However, further studies with more age-groups and animals are needed to confirm our observations on the effects of ACTH treatment.

Results from other studies have shown neonatal decreases in protease activity from pancreatic tissue (Weström et al., 1987), intracellular dipeptidase activity (Lindberg & Karlsson, 1970) and some mucosal transport parameters (Henriques de Jesus & Smith, 1974) when newborn pigs were allowed access to sow's milk (colostrum). These observations were explained by sucking-dependent secretion of pancreatic enzymes and changes in the mucosal structure resulting from the first exposures of the mucosa to nutrients (colostrum). In the present study the activities of pancreatic enzymes did not decrease within the first week (Figure 2) but most of the intestinal enzyme activities (Figure 1) appeared to have higher values at birth (0-1 days) than a few days later (3 and 5-7 days). Two of the pigs in the 0-1 day group had particularly high activities of all enzymes and this may be explained by a shorter period of fasting for these pigs (8 hours) compared with the other pigs in that group (approximately 24 hours). In mature animals fasting and feeding are known to either decrease or increase enzyme activities depending on the enzyme, the diet and the length of the fasting period (Alpers, 1987). However, the effects of fasting and feeding could be different in newborn unsuckled animals. Further studies are needed elucidate the role dietary and hormonal factors in the postnatal development of intestinal and pancreatic enzymes in the young pig.

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ABSORPTION OF FREE OR PROTEIN-BOUND LYSINE AND THREONINE IN CONSCIOUS MULTICANNULATED PIGS

J. T. Yen[1], R. A. Easter[2] and B. J. Kerr[2]

[1]U.S. Department of Agriculture, Agric. Res. Service, R. L. Hruska, U.S. Meat Animal Research Center, Clay Center, NE, U.S.A.
[2]University of Illinois, Urbana, IL, U.S.A.

Abstract

Six gilts, weighing 48 kg, having chronic cannulas placed in the hepatic portal vein, carotid artery and ileal vein and trained to consume once daily 1.2 kg of feed, were used in a cross-over design employing a 16% CP corn-soybean meal diet and a 12% CP corn-soybean meal diet supplemented with free lysine, threonine and tryptophan to levels equal to those contained in the 16% CP diet. Based on the appearance of peak concentrations of plasma lysine and threonine in the portal or arterial samples and peak net absorption of lysine and threonine into the portal vein, it is concluded that free lysine and threonine are absorbed more rapidly than protein-bound lysine and threonine by pigs after feeding.
Keywords: portal vein, absorption, lysine, threonine, pigs.

Introduction

More and more free, crystalline amino acids (AA) are used to supplement diets for pigs. The efficiency of utilization of free lysine in diets for pigs, however, is affected by feeding frequency. When diets supplemented with free lysine were fed to pigs once daily than six or three times a day, live weight gain, carcass gain, feed conversion or nitrogen retention of pigs were reduced (Batterham, 1974, Batterham & O'Neill, 1978; Batterham & Murison, 1981, Cook et al., 1983, 1985). Using [^{14}C] phenylalanine as an indicator to monitor amino acid metabolism, Batterham & Bayley (1989) observed greater oxidation of labelled phenylalanine by pigs fed once daily a diet containing free lysine. These authors suggested that there was a difference in the rate of absorption between free and protein-bound lysine by pigs fed once daily and that an imbalance of amino acids occurred at the sites of protein synthesis as a result of more rapid absorption of free lysine. However, direct measurement of the absorption of lysine was not conducted.

The objective of the present study was to measure directly the absorption of lysine and threonine into the hepatic portal vein in pigs fed a diet containing protein-bound lysine and threonine or both free and protein-bound lysine and threonine.

Materials and Methods

Chronic cannulas were placed in the hepatic portal vein, carotid artery and ileal vein of six growing crossbred (Chester White x Landrace x Large White x Yorkshire) gilts, trained to consume once daily 1.2 kg of a reference diet mixed with 1.2 liter of water at

0930. The reference diet contained 76.3% corn, 19.6% soybean meal (44% CP), 2.4% dicalcium phosphate, 0.5% limestone, 0.4% iodized salt, 0.4% trace mineral premix, 0.2% vitamin premix and 0.2% choline chloride. Detailed information regarding the construction of the cannulas and procedures of surgery has been reported previously (Yen and Killefer, 1987; Yen et al., 1989). After the pig had regained its preoperation appetite for at least 7 days, it was weighed 6 h postprandially and placed into a rectangular metabolism cage. On the following day, the pig was assigned to receive either a test 16% CP corn-soybean meal diet (16% CP) or a 12% CP corn-soybean meal diet supplemented with free lysine, threonine and tryptophan (12% CP + AA) to the levels equal to those contained in the test 16% CP diet (Table 1).

Table 1. Composition of test diets.

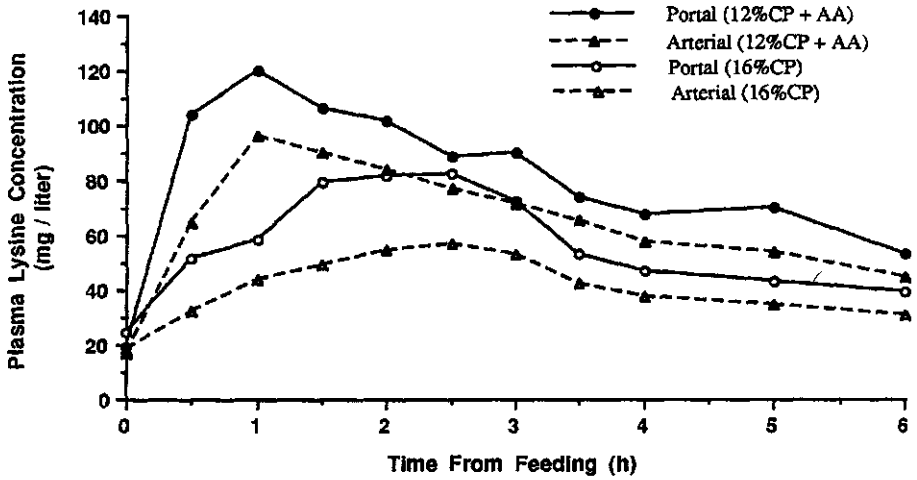
Item	Diets	
	16% CP	12% CP + AA
Ingredients, %		
Corn	76.90	86.16
Soybean meal, 48% CP	20.65	10.80
Dicalcium phosphate	0.91	0.97
Limestone	1.09	1.03
Trace mineral mix	0.35	0.35
Vitamin mix	0.10	0.10
L-lysine-HCl	---	0.35
DL-tryptophan	---	0.08
L-threonine	---	0.16
Analyzed composition		
Dry matter, %	88.8	88.3
CP, %	15.7	12.3
Calculated composition[a]		
Lysine, %	0.83	0.83
Threonine, %	0.67	0.67
Tryptophan, %	0.19	0.19

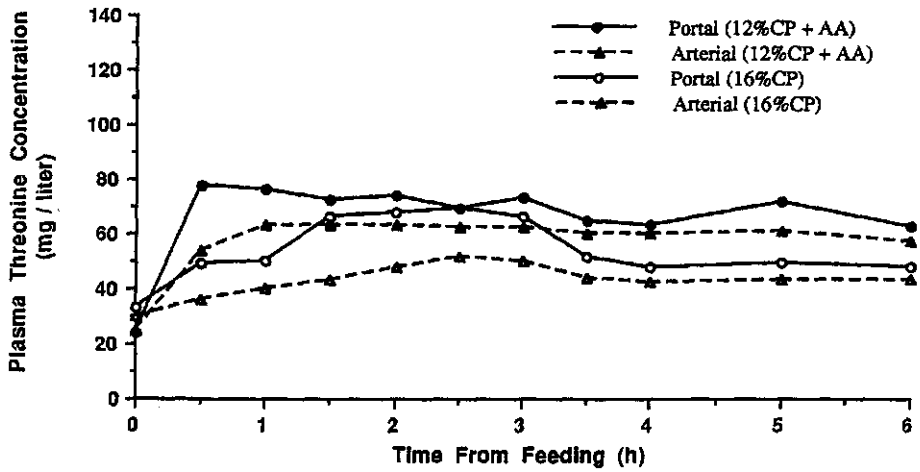
[a]Based on analyzed amino acid composition of corn and soybean meal.

The 1.2-kg test diet was mixed with 1.2 liter of water and given to the pig at 0930. Portal and arterial blood samples were obtained simultaneously before feeding, once every 30-min during the first 4 postprandial h and hourly during the 5 to 6 postprandial h. The plasma concentrations of amino acids were determined by ion-exchange chromatography. The net portal absorption of lysine or threonine was calculated by multiplying the porto-arterial plasma concentration difference of the amino acid by the portal plasma flow rate. The portal plasma flow rate was estimated by an indicator-dilution technique employing p-aminohippuric acid as the indicator and infused into the ileal vein (Yen & Killefer, 1987; Yen & Pond, 1990).

After the first sequence of measurements, the pig was returned to

Figure 1. Portal and arterial plasma concentrations of lysine and threonine in pigs fed a diet containing protein-bound lysine and threonine (16%CP) or a diet containing both free and protein-bound lysine and threonine (12%CP + AA).





its home pen and fed the reference diet once daily for 2 d. It was then weighed 6 h postprandially and again placed into a rectangular metabolism cage. On the following day, the pig was fed the other test diet at 0930 and the second sequence of measurements were conducted. The pigs weighed 47.7 ± 1.6 and 48.0 ± 1.3 kg, respectively, when they were fed the 16% CP test diet and the 12% CP + AA diet.

The data were analyzed as a cross-over design with the GLM procedure of SAS (1989). Type III sum of squares was used for data analysis.

Results and Discussion

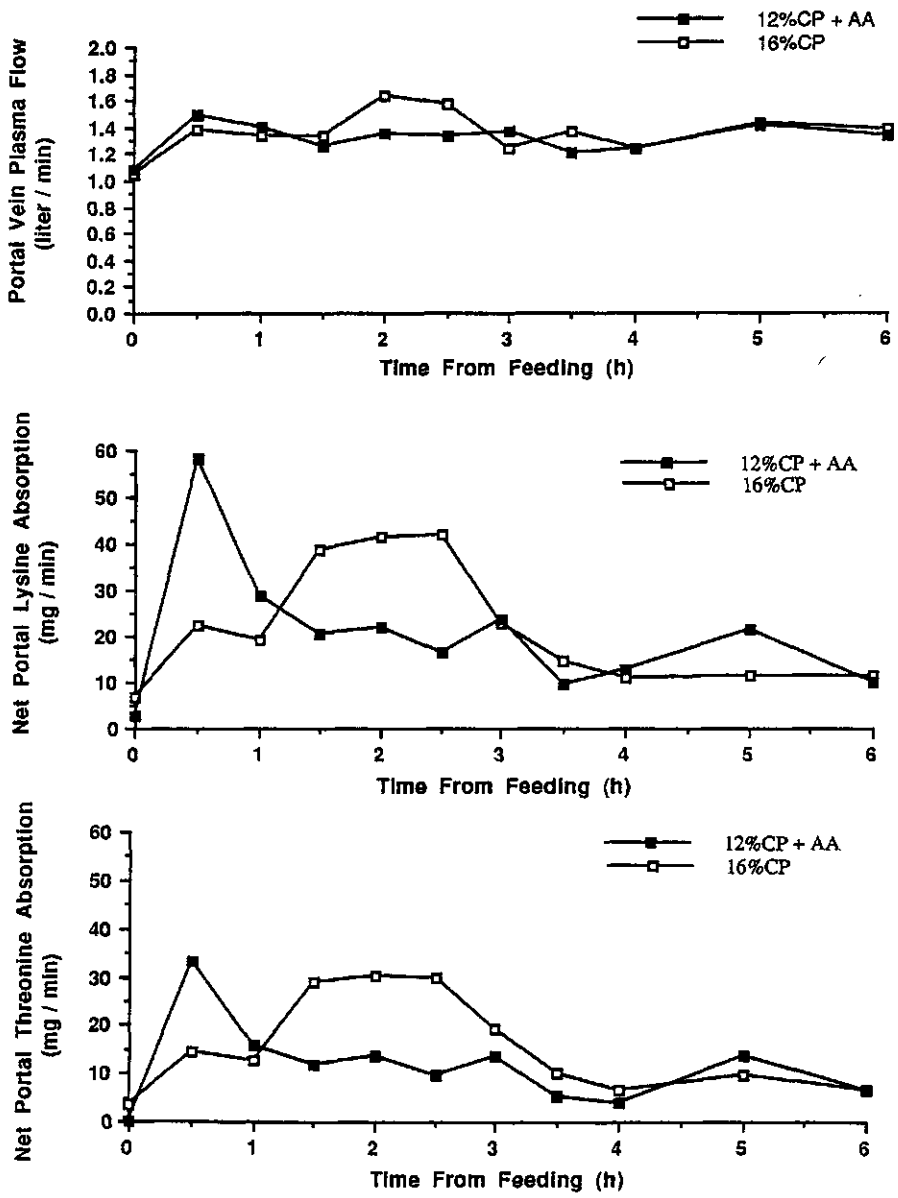
As shown in Figure 1, both the plasma lysine concentrations in the portal and arterial samples rose after feeding and attained the maximum level at 1 h postprandially when pigs were given the 12% CP + AA diet, containing both free and protein-bound lysine, threonine and tryptophan. Whereas, when pigs were fed the 16% CP diet containing protein-bound AA, the portal and arterial plasma lysine concentrations reached the peak level at 2.5 h postprandially. During the 0.5- to 1.5-h and at 6-h postprandial periods, the rise of portal plasma lysine concentration was greater ($P<0.05$) when the 12% CP + AA diet than the 16% CP diet was fed. Likewise, during the 0.5- to 3-h postprandial period, the arterial plasma lysine concentration was greater ($P<0.05$) when pigs were given the 12% CP + AA diet than the 16% CP diet.

The portal plasma threonine concentration rose to the maximum level at 0.5 h after feeding when pigs were given the 12% CP + AA diet but at 2.5 h postprandially when they were fed the 16% CP diet. During 1-h and 5-h postprandial periods, the rise of portal plasma threonine concentration was greater ($P<0.05$) when pigs were given the 12% CP + AA diet than the 16% CP diet. The arterial plasma threonine concentration reached the peak level at 1.5 h after feeding when pigs were given the 12% CP + AA diet but at 2.5 h postprandially when the 16% CP diet was fed. During the 0.5- to 4-h postprandial period, the arterial plasma threonine concentration was greater ($P<0.05$) when pigs were given the 12% CP + AA diet than the 16% CP diet.

The portal vein plasma flow rate of pigs during the 6-h postprandial period is depicted in Figure 2 and showed no difference ($P>0.05$) whether pigs were given the 12% CP + AA diet or the 16% CP diet. When the portal vein plasma flow rate was multiplied by the porto-arterial concentration difference of lysine or threonine to calculate the net absorption of the amino acid into the portal vein, the peak portal absorption of lysine and threonine appeared at 0.5 h after feeding when pigs were given the 12% CP + AA diet but at 2.5 h postprandially when they were fed the 16% CP diet.

Because the 12% CP + AA diet and the 16% CP diet were calculated to contain the same amounts of lysine and threonine, the earlier appearance of peak portal concentrations of plasma lysine and threonine and the peak net portal absorption of lysine and threonine in pigs when they were given the 12% CP + AA diet than the 16% CP diet would suggest that the free lysine and threonine in the 12% CP + AA diet were absorbed and transported into the portal vein more rapidly than the protein-bound lysine and threonine in the 16% CP diet.

Figure 2. Portal vein plasma flow rate and net portal absorption of lysine and threonine in pigs fed a diet containing protein-bound lysine and threonine (16%CP) or a diet containing both free and protein-bound lysine and threonine (12%CP + AA).



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AMINO ACID ABSORPTION AND ITS SIGNIFICANCE FOR PROTEIN SUPPLY IN THE PIG

M. Schmitz [1], F. Ahrens [1], Jutta Schön [1], H. Hagemeister [2]

1 IS Forschungsgesellschaft für experimentelle Tierphysiologie und Tierernährung, Wahlstedt, Germany
2 Institut für Physiologie u. Biochemie der Ernährung, Bundesanstalt für Milchforschung, Kiel, Germany

Abstract

The significance of a possible absorption of amino acids in the large intestine for protein supply of the pig was investigated. Homoarginine (HA) was used as a model as naturally HA is detected only in traces in blood. Therefore its absorption can be verified by detection in blood. HA was infused into the caecum or was fed in the same dose at only 10 % and 5 % of this dose as HA-containing casein. Infusion and feeding of HA-containing casein were followed by determination of HA concentration in blood.
After infusion of HA-containing casein into the caecum (0,36 mmol HA per (kg bodyweight) ^{0,75}) no HA was detectable (detection limit: 0.005 µmol/ml) at any time of blood sampling (0, 1, 5 and 24 h after start of infusion). In contrast to this high concentrations of HA were determined in blood after feeding the same amount of HA-containing casein (1 h postprandial: 0.085 µmol/ml, 5 h postprandial: 0.121 µmol/ml, 24 h postprandial: 0.096 µmol/ml). Also after feeding only 10 % of the amount of casein infused into the caecum HA was clearly detected in blood (1 h postprandial: 0.029 µmol/ml, 5 h postprandial: 0.039 µmol/ml, 24 h postprandial: 0.031 µmol/ml). After feeding 5 % of the dose infused into the caecum there was no HA detectable in blood. The results show that if any amino acids were absorbed in the large intestine the percentage is less than 10 % of the amount infused into caecum and less than 3 % of the protein maintenance requirement of the pig. It is concluded that the absorption of amino acids in the large intestine is insignificant for protein supply in the pig.

Introduction

Significant amounts of protein and amino acids are present in the large intestine of the pig. In principle amino acids can be absorbed in the colon (Niiyama et al. 1979) and caecum (Olszewski and Buraczewski, 1978) of the pig. On the other hand Zebrowska (1975) demonstrated that 83 % of the nitrogen from hydrolyzed casein infused into the terminal ileum was excreted with urine. This shows the degradation of amino acids to ammonia and amines, which are absorbed and excreted with urine. Just et al. (1981) indicated that lysine, which was infused into the caecum, was not absorbed markedly. In the present investigation the question of amino acid absorption in the large intestine should be answered using the amino acid homoarginine (HA). HA is detected naturally only in traces in blood (Kato et al., 1989). Therefore the absorption of even small amounts of HA are detectable. HA-concentration in blood after infusion of HA-containing casein into the caecum is related to HA-concentration in blood after feeding 100%, 10 % and 5 % of the amount of HA-containing casein infused into the caecum.

Materials and Methods

In all parts of the experiment HA was given as HA-containing casein (0,460 mmol HA per g casein). HA was formed in casein from lysine by guanidination. Guanidination was performed with a 0,2 M O-methyl-isourea solution at pH 10.5 and 4° C for 96 h (Hagemeister and Erbersdobler, 1985). Four adult Göttingen miniature pigs (bodyweight 50 - 60 kg) with T-cannula at the caecum were used in the experiment. In the first part of the experiment 0,36 mmol HA per (kg bodyweight) ^{0,75} were infused into the caecum of the animals in five equal parts from 8.00 a.m. to 13.00 p.m. In three further independent parts of the experiment the pigs were fed with the morning meal 0,36, 0,036, and 0,018 mmol HA per (kg bodyweight) ^{0,75}, corresponding to 100 %, 10 % and 5 % of the amount infused into the caecum. Between each part of the experiment there was an adaption period of two weeks. Before start of infusion or feeding of HA-containing casein and after 1, 5 and 24 h HA-concentration in blood was determined. Blood serum samples were centrifuged (4000 xg for 10 min). 4 ml serum were deproteinized with 1 ml sulfosalicylic acid (0,78 mol/l) and centrifuged again as described above.

Results and Discussion

HA-concentration in blood serum of minipigs following caecal infusion or feeding of HA-containing casein is shown in table 1.

Table 1. Homoarginine (HA) concentration in blood serum of Göttingen miniature pigs after caecal infusion or feeding of HA-containing casein (values are means +/- s, n = 4 animals).

Time after start of infusion or feeding	caecal infusion 0,36 mmol per (kg bodyweight) ^{0,75}	feeding 0,36 mmol per (kg bodyweight) ^{0,75}	feeding 0,036 mmol per (kg bodyweight) ^{0,75}	feeding 0,018 mmol per (kg bodyweight) ^{0,75}
h	µmol/ml	µmol/ml	µmol/ml	µmol/ml
0	n.d.	n.d.	n.d.	n.d.
1	n.d.	0,085 +/-0.020	0,029 +/-0,028	n.d.
5	n.d.	0,121 +/-0,021	0,039 +/-0,016	n.d.
24	n.d.	0,096 +/-0,027	0,031 +/-0,021	n.d.

n.d. = not detectable, dectection limit: 0,005 µmol/ml

Before start of infusion or feeding no HA was dectectable. After feeding 0,36 mmol HA per (kg bodyweight) ^{0,75} 1, 5 and 24 h postpandrially HA-concentration in blood serum was 0,085, 0,121 and 0,096 µmol/ml respectively. After feeding 10 % of this dose (0,036 µmol (per kg bodyweight) ^{0,75} HA-concentration 1, 5 and 24 h postpandially was 0,029, 0,039 and 0,031 µmol/ml blood serum, respectively. When the amount of HA fed was reduced to 0,018 mmol per (kg bodyweight) ^{0,75} no HA was found in blood serum at any time of taking blood samples. After infusion of HA-containing casein into the caecum no HA was detectable in blood serum at any time of blood sampling.
Former investigations (Schmitz, 1988) showed a rapid rise of postprandial HA-concentration in blood following intake of HA-containing casein. This proofs the fast proteolysis of the protein and the absorption of HA from

the small intestine. Zebrowska (1975) and Just et al. (1981) demonstrated the high proteolytic capacity of the large intestine measuring a nearly complete degradation of casein infused into the caecum. Therefore it can be expected that HA was liberated in the large intestine after the caecal infusion of HA-containing casein and was avaiblable for absorption in the large intestine. After infusion of HA-containing casein into the caecum no HA was detected in blood. In contrast to this feeding only 10 % of the dose infused into the caecum led to marked HA-concentrations in blood. As after feeding 5 % of the dose infused into the caecum there was no HA detectable in blood it can only be concluded that the amount of HA possibly absorbed in the large intestine is less than 10 % of the amount infused. The HA-containing casein infused into the caecum amounted to 30 % of the protein maintenance requirement of the pigs. Therefore, if any amino acids were absorbed from the large intestine the amount was less than 3 % of the maintenanece requirement of the pigs. The results are in accordance with results of Zebrowska (1975) and Just et al. (1981), who showed that the absorption of amino acids in the large intestine is insignificant for protein supply.

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THE KINETICS OF PLASMA LYSINE AFTER ORAL AND PARENTERAL ADMINISTRATION TO PIGS

J.M. Fentener van Vlissingen[1*], J.G.M. Bakker[1], L.P. Jager[2], N.P. Lenis[1], H. de Visser[1], F.G.M. Russel[3], J.E. van Dijk[2]

[1]Research Institute of Livestock Nutrition, P.O. Box 160, 8200 AD Lelystad, The Netherlands

[2]Central Veterinary Institute, Lelystad, The Netherlands
[3]Department of Pharmacology, Faculty of Medicine and Dentistry, University of Nijmegen, The Netherlands

* Present address:
TNO Institute of Animal Nutrition and Animal Physiology, P.O. Box 15, NL 6700 AA Wageningen, Netherlands.

Abstract

The fate of lysine, administered to growing pigs (± 45 kg live weight) via oral or parenteral routes, was quantified by pharmacokinetic analysis of systemic plasma concentrations. Elimination rate after rapid intravenous administration had a half-life of 2.0 ± 1.6 hrs. Absorption of an oral dose of lysine from the intestine (half-life of 3.6 ± 0.9 hrs) was slower than elimination (half-life of 0.9 ± 0.3 hrs). No significant liver first-pass effects could be detected and bioavailability of lysine after enteral uptake was found to be 82 ± 6%.
Analysis of plasma kinetics of amino acids may provide a valuable tool to evaluate bioavailability of dietary amino acids. Furthermore, it can be used to quantify absorption rate, elimination rate, and intestinal or hepatic metabolism.

Introduction

The growth of animal tissues depends on the availability of substrates and a complex of growth regulatory mechanisms (Buttery & Lindsay, 1980). Animal nutrition research typically studies the supply of substrates by feed. The maximal attainable bioavailability of substrates for tissue growth is reflected by the digestibility of nutrients from feed, as measured by the disappearance from digesta (Rerat, 1985; Sauer & Ozimek, 1986). The bioavailability depends also on the metabolic economy of intestinal microflora, and enteral and hepatic first-pass metabolism.
The concentration of substrates in the systemic blood is determined by: 1. increase kinetics, dependent on gastrointestinal and liver processing of nutrients (Rerat, 1985); 2. degradation products from tissue catabolism (Edmonds et al., 1987; Kimura et al., 1987); 3. elimination kinetics determined by metabolism in many different tissues (biosynthesis, oxidation) and 4. excretion of the substrate

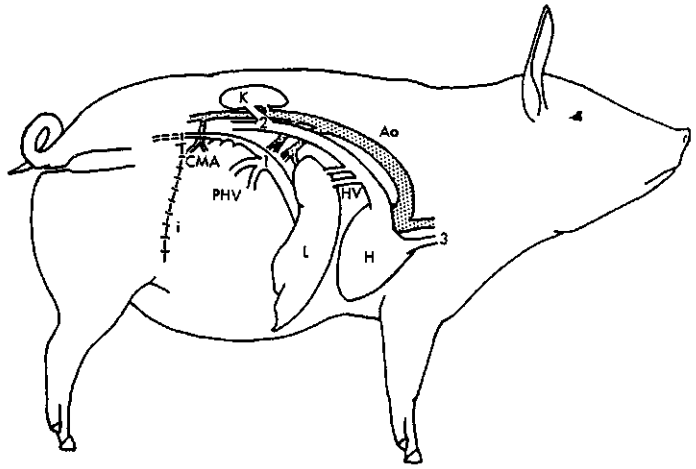
or its metabolites (Condon, 1986; Tsuchiya et al., 1989). Although the mechanisms, which influence the kinetics of blood concentrations, are extremely complex (Istfan et al. 1988), the blood concentrations, as measured directly, are relevant for the availability of nutrients at the tissue level. Pharmacokinetic analysis provides parameters on uptake rate, total bioavailability, elimination rate, and putative first-pass effects. Quantitative parameters may be instrumental to indicate, though not identify, different metabolic routes.
The present experiment was designed to determine the kinetics of plasma lysine after oral, portal and systemic administration. The applicability of a pharmacokinetic approach to the evaluation of bioavailability of nutrients is discussed.

Materials and Methods

2.1 Animals

Three pigs (cross-bred of Dutch Landrace * Large White), aged 15 weeks, were housed individually in pens. Catheters (refer to Fig. 1 for catheter positioning) were placed surgically while the animal was under general inhalation anesthesia.

FIGURE 1
Schematic drawing of position of portal, caval and jugular catheters and surgical access to the abdominal cavity.
Shaded vessels are arteries and open vessels are veins (portal or systemic).
Abbreviations: Ao = aorta; CMA = caudal mesenteric artery; H = heart; HV = hepatic veins, draining into the caudal caval vein; i = incision; K = Kidney; L = liver; PHV = portal hepatic vein.
Location of tip of catheters: 1 = portal; 2 = caval; 3 = jugular.



The caudal mesenteric vein was approached by a vertical incision in the right flank and exposed by blunt dissection of the descending mesocolon. The catheter (Tygon, ID 1.0 mm, OD 1.8 mm, from Talas) was introduced into the vein and advanced cranially over a distance of approximately 12 cm, corresponding to a position of the tip near the junction of the caudal mesenteric and cranial mesenteric veins. The blood from the caudal portion of the vein drained to the rectal veins via preexistent anastomoses without any apparent obstructions. The catheter was exteriorized at the back. In one out of three animals, the sham-operated control, the catheter was introduced into a lumbar branch of the caudal caval vein, within the same region of the abdomen. The left deep jugular vein was subsequently catheterized similarly and exteriorized at the right dorsal aspect of the neck. Catheter exteriorization sites were decontaminated with 70% (v/v) ethanol and covered with adhesive bandage.

No antibacterial or analgesic therapy was indicated. Catheters were flushed daily with physiological saline containing 250 IU heparin per ml. Slow intravenous infusions were administered through a disposable IV-infusion device. Rapid infusions were performed with 60 ml syringes. Heparinized blood samples (4 ml) were each immediately transferred to a centrifuge tube cooled on ice. After centrifugation (1600 * g, 10 minutes, 4°C) the plasma was transferred to microvessels and frozen at -20°C prior to deproteinization.

After completion of the series of experiments each animal was killed by an intravenous injection of barbiturate (3 to 4 g of Sodium Pentobarbital. Immediately prior to injection of the barbiturate a 20% (v/v) suspension of Indian Ink in saline was injected into the portal/caval catheter for postmortal evaluation of its intravital position. Macroscopic postmortem examination included the surgical sites, organs, and evaluation of the position of the catheters. The presence of ink particles in systemic and portal blood samples was estimated in a semi-quantitative manner after centrifugation of a dilute (10%) blood sample after hypotonic emolysis. Hepatic tissue was examined histopathologically.

2.2 Experimental Procedures

Feed was given at a level just exceeding animal maintenance requirements, from the day of surgery onwards. This was aimed to prevent a substantial increase in body weight during the experimental period and to ensure rapid intake of the daily ration, when this was offered. Protein content of the diet (16.1%) was rather low in order not to obscure blood lysine concentrations by high lysine levels derived from the feed. In order to maximize the utilisation of dietary amino acids for protein synthesis, dietary amino acid supply did hardly exceed the need for maintenance. Energy content of the diet (2150 kCal net energy per kg

feed) was somewhat higher as compared to protein, in order to minimize the oxidation of protein as a source of dietary energy. The feed was composed as follows: 77.7% barley, 19.4% tapioca, 1.5% calcium hydrogen phosphate, 0.7% calcium carbonate, 0.25% sodium chloride, 0.20% vitamin/mineral supplement, 0.15% L-threonine, 0.10% DL-methionine. The animals were given 48 g of the experimental feed per kg metabolic weight ($kg^{0.75}$) at 8 a.m.. This amount was saturated with water 20 minutes before feeding. Most of the meal was finished within half an hour. The animals had free access to water.

Crystalline L-lysine (Fluka Chemie AG) was administered at a daily dose of 0.53 g/kg metabolic body weight ($kg^{0.75}$), corresponding to 62 or 63 mmol per animal weighing 44 or 45 kg respectively, and was prepared for oral or intravenous administration as follows. Lysine was dissolved in distilled water at an initial concentration of 150 g/L, yielding a pH of approximately 10.1. The pH was adjusted to 7.5 with a 5 M HCl-solution. The solution was then sterilized over a 20 μm cellulose acetate sterile filter unit (Nalge Co.) and diluted 3 times, yielding a slightly hypertonic solution as compared to plasma. The solutions for intravenous administration were brought to 37°C immediately before use.

L-lysine was administered to each animal in four different ways on consecutive days and then the treatments were repeated once. Within each series the order of the different treatments was randomized. The oral dosage was included in the meal, provided at 8 a.m., and intravenous infusions included slow jugular infusion from 9 to 10 a.m., rapid jugular infusion from 9.00 to 9.10 a.m. and slow portal/caval infusion from 9 to 10 a.m.. When lysine was given orally a jugular intravenous dose of 300 ml saline was infused from 9 to 10 a.m.. Blood sampling was done at 8 a.m., at 9 a.m., at 9.10 a.m. (treatment C only), every 30 minutes up to 11 a.m., at noon, and at 2, 4, and 6 p.m..

2.3 Amino Acid Analysis

Heparinized plasma samples were prepared for amino acid analysis according to Condon (1986) with some slight modifications. Norleucine was added as an internal standard. Amino acid concentration was measured by chromatography on a LKB 4151 Alpha Plus amino acid analyzer with the recommended sodium citrate buffer system. Validation of the method included evaluation of storage effects and recovery of lysine added to blood/plasma samples. Recovery of lysine added to plasma ranged from 91 to 106% and recovery from heparinized blood ranged from 108 to 122%, indicating that the uptake of lysine by blood cells during 20 minutes equilibration at room temperature was less than proportional to equilibration in the plasma compartment.

2.4 Data Analysis

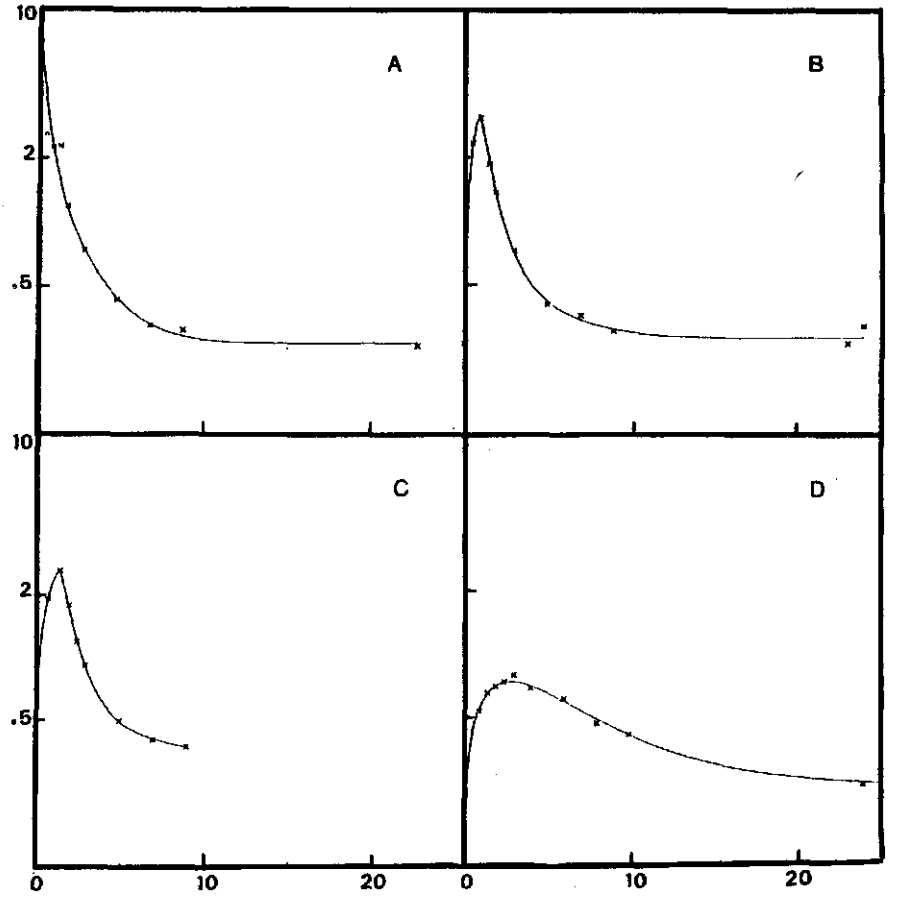
Analysis of kinetic parameters of lysine absorption and elimination was done by three different models, dependent on the best mathematical description of the rough data. Plasma concentration-time curves of lysine after intravenous bolus injection or slow infusion could be described best by a two-compartment model with elimination from the central compartment. The curves obtained after oral administration were adequately described by a one-compartment model with first-order absorption. For standard equations to these models, see Gibaldi & Perrier, 1982. To account for the endogenous lysine levels in plasma, the basal plasma level (C_B) was added to each of the equations used. Plasma levels of each subject were fitted to the appropriate models using the nonlinear least squares regression program NONLIN (Metzler et al., 1974), in which all data were reciprocally weighed ($1/C^2$). From these fits basic pharmacokinetic parameters were calculated.

Results

Recovery from surgery by the animals and subsequent experiments were uncomplicated. The animals were never feverish and somatic growth (increase of body weight, as assessed after an overnight fast) during the experimental period ranged from 1 to 4 kg and corresponded to the restricted feeding regimen. Gross aspect and weight of organs provided no indication of functional disturbances. Catheter position as evaluated by dissection and recovery of ink particles indicated that two animals (P1 and P2) had the catheter in the portal venous system with the tip at the junction with the cranial mesenteric vein, and one animal (C) had the catheter in caval position. This control animal proved valuable with respect to the causal explanation of hepatic changes, as detected in the portally catheterized animals, but not in the cavally catheterized animal. These changes of the liver were characterized by cellular swelling, associated with extensive glycogen storage and causing partial occlusion of the liver sinusoids.

Elimination kinetics after rapid intravenous (jugular) infusion of lysine were best described by a two-compartment model. The deduced kinetic parameters are presented in Table 1 and an example of plasma lysine concentration as a function of time, and fitted curve, is shown in Figure 2A. The data indicate that lysine was eliminated quite rapidly from the plasma under these circumstances. Initial volume of distribution averaged 0.18 l/kg while the volume of distribution at steady state was 0.43 L/kg. Thus, after an initial rapid distribution over the plasma (blood) compartment, there is some expansion to a second, larger compartment, including other extracellular and intracellular fluid compartments.

FIGURE 2.
Plasma lysine concentration (Y-axis, logarithmic, mmol/L) in relationship to time (X-axis, linear, hours) after administration of 0.53 g/kg$^{0.75}$ of lysine via different routes and at different rates. Typical examples of each treatment (animal P1) are shown, presenting both the rough data and the curves fitted according to the models referred in the Materials and Methods section. Refer to Table 1 for pharmacokinetic parameters.
A: jugular bolus injection; B: slow jugular infusion; C: slow portal infusion; D: oral administration.



Kinetics of plasma lysine concentration after slow intravenous (jugular, caval, or portal) infusion were also analysed by a two-compartment model, but infusion and elimination kinetics were calculated separately (Table 1). An example of plasma concentration after jugular infusion, as a function of time, and fitted curve, is presented in Figure 2B. The data obtained after portal infusions (Fig. 2C) were similar to those found after jugular or caval infusion. The area under the curve (AUC) ranged from 4.24 to 6.54 mmol/hr*L after infusion into a systemic vein and from 3.85 to 5.29 mmol/hr*L after portal infusion, indicating that no detectable liver first-pass effects were present. Mean residence time (MRT) and clearance (CL) after slow intravenous infusion are similar to these parameters after jugular bolus injection as well. Therefore, the data obtained after slow intravenous infusion by either route were combined in Table 1. Distribution volume in the central compartment tends to be somewhat larger after slow intravenous infusion than after jugular bolus injection, indicating progression to steady state distribution during the infusion period.

TABLE 1.
Pharmacokinetic parameters of lysine after intravenous (jugular) bolus injection, intravenous infusion (jugular or caval), and oral administration. Mean ± SD.

pharmaco-kinetic parameter	**intravenous bolus** (n = 6)	**intravenous infusion** (n = 8)	**oral administration** (n = 6)
F	1.00	0.91 ± 0.16	0.82 ± 0.06
CL	0.24 ± 0.03	0.25 ± 0.04	0.24 ± 0.04
V_1	0.18 ± 0.02	0.26 ± 0.03	---
V_{ss}	0.43 ± 0.25	0.62 ± 0.22	0.29 ± 0.10
MRT	1.9 ± 1.4	2.6 ± 1.2	6.5 ± 1.4
MAT	---	---	4.5 ± 1.8
$t_{1/2,z}$	2.0 ± 1.6	3.7 ± 1.9	0.9 ± 0.3
$t_{1/2,A}$	---	---	3.6 ± 0.9
C_B	0.24 ± 0.02	0.23 ± 0.03	0.22 ± 0.03

Abreviations and units: F = bioavailability; CL = clearance (L/hr*kg); V_1 = initial volume of distribution (L/kg); V_{ss} = volume of distribution at steady-state (L/kg); MRT = mean residence time (hr); MAT = mean absorption time (hr); $t_{1/2,z}$ = half-life of the terminal exponential phase (hr); $t_{1/2,A}$ = half-life of the absorption phase (hr); C_B = basal lysine concentration (mmol/L).

The kinetics of plasma lysine concentration after oral administration are presented in Table 1 and Figure 2D. After oral administration of lysine the concentration in the systemic blood increased with a time constant (τ_{abs}) of 3.8 to 7.6 hours and an absorption half-life of 2.6 to 5.3 hours (Table 1), indicating that absorption from the intestine takes several hours and is dependent on absorption kinetics rather than enzymatic digestion, which clearly is not an essential step in the digestion of lysine monomers. The oral uptake of lysine derived from the basal ration is negligible as compared to the experimental oral dose and was present during comparative intravenous dosage schedules and therefore treated as an endogenous level in data analysis. Elimination kinetics of lysine after an oral dose were similar to elimination kinetics after intravenous administration (Table 1).
Bioavailability of lysine after an oral dose was deduced from kinetic parameters and presented in Table 1. The mean area under the curve after oral administration in each animal was divided by the mean area under the curve after intravenous bolus injection in the same animal and expressed as the fraction absorbed. The oral bio-availability was 82 ± 6%.

Discussion

The present experiment was designed to explore the potential of a pharmacokinetic approach to determine amino acid absorption and metabolism (bioavailability) by analysis of changes in plasma concentrations.

The hepatic changes observed in those animals that had been infused with lysine through the portal vein, contrasting with the control animal that had been infused intracavally, remain unexplained. No direct hepatotoxic effects from lysine have been documented so far, and ionic strength and pH of the solution were not likely to cause any tissue damage when infused at a rate so slow. The intravenous load of lysine per se did not cause any excessive hepatic glycogen storage in the control animal, so hepatic changes were apparently associated with the portal route of introduction and may depend on a regional endo-/paracrine mechanism. The distribution of lysine over blood plasma and cellular compartments, in vivo and in vitro, indicated that lysine initially occupied the extracellular fluid compartment. Later, amino acids including lysine can be enriched in tissues and blood cells as compared to plasma (Edmonds & Baker, 1987; Van Berlo, 1988).

The quantification of enteral uptake of lysine by plasma kinetic parameters seems to be suitable when various routes of administration are compared. The enteral absorption kinetics show that uptake occurred at a substantial rate during several hours after intake, starting quite rapidly. Half of the lysine was absorbed after three to five hours.

Enzymatic hydrolysis of protein, often thought to be the rate limiting step in protein digestion, did not play a role in this model and it is thus concluded that enteral absorption was the rate limiting step in the process of transfer of lysine to the systemic pool. This corresponds to the observations of Buraczewski et al., 1980, who measured the rate of disappearance from the digesta directly. The rate of absorption corresponded well to portal absorption data obtained by Rerat (1985).

Elimination of plasma lysine occurred at a similar rate after each route of administration. This does not proof, but suggest, that elimination mechanisms involved may be identical in each case. Apparently, elimination rate was not enhanced when plasma concentrations of lysine were excessive. Elimination of lysine from plasma may occur in different ways, for example conversion to other amino acids by reduction of the part of the molecule that is specific to lysine, by desamination and/or decarboxylation, by oxidation (Imura & Walser, 1988; Reiser et al., 1988; Jahoor et al., 1988), by excretion, or by incorporation into peptides/proteins. The incorporation of labeled lysine administered orally or intravenously in human milk proteins was somewhat faster after intravenous bolus injection but cumulative incorporation after several hours was identical (Irving et al., 1988). Urinary excretion may be a function of plasma concentration (Tsuchiya et al., 1989).
Hepatic metabolism seemed to be quantitatively insignificant, while no liver first-pass effect was detected by the methods used. The bioavailability of an oral dose of lysine was high, indicating that enteral metabolism is limited. Digestibility of synthetic lysine was not quantified in this experiment, but generally free amino acids added to the feed have disappeared by 100% from the digesta when these arrive at the end of the small intestine (Sauer & Ozimek, 1986). The amount of 18% of the lysine that is not available to the animal corresponds well to the proportion metabolized by the intestinal microflora, as reported by Dierick et al. (1986a & 1986b).

Although amino acids may not be stored by the animal as effectively as fat or glucose, the slow characteristics of absorption and elimination kinetics account for a circulating pool that well covers the usual between-meals intervals. During extended intervals between meals (24 hours in this study) tissue proteins are degraded to provide adequate levels of circulating amino acids.
The approach of quantification of bioavailability of nutritional amino acids by studying plasma kinetics may be a useful tool in the prediction of nutritional value of feeds and feedstuffs, when the limitations of the method are respected. Firstly, not all amino acids show a distinct postprandial peak (Fentener van Vlissingen et al., 1990). It seems that only the uptake of amino acids with distinct changes in postprandial plasma concentrations can be evaluated this way. Secondly, although this experiment on

lysine did not reveal a hepatic first-pass effect, this does not predict such an effect to be absent with respect to other amino acids to be studied (Rerat, 1985). The research on functional plasma amino acid levels may also lead to the definition of desirable (recommended) plasma amino acid patterns in relationship to growth. This would provide a method to evaluate feeds, but also feeding strategies, with respect to bioavailablity of dietary protein for animal production. It is, however, a field that has hardly been explored yet and that only comes within view by the introduction of more efficient methods of quantification of amino acids.

Acknowledgements

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ANTIBODIES FORMATION AGAINST PEA PROTEINS IN PIGLETS

M.P. Le Guen[1], G.H. Tolman[2] and J. Huisman[2]

1 G.I.E. EURETEC, 12 avenue George V, 75008 Paris, France
2 TNO-Institute of Animal Nutrition and Physiology (ILOB), PO Box 15, 6700 AA Wageningen, The Netherlands

Abstract

In young piglets given a raw pea-based diet, circulatory antibodies developped against pea legumin and vicilin. The maximum was reached in about 3 weeks after commencement of experimental feeding. The initial and thereafter antibody levels were higher when the mother's diet contained peas.

Introduction

In normal physiological conditions, the gut-wall prevents the passage of dietary antigens from the intestinal lumen into the blood circulation (Walker et al. 1975). In preruminant calves fed raw pea flour, Nunes do Prado et al. (1988) however observed a formation of antibodies against pea protein, along with an increased gut permeability to macromolecules.
The objective of the present study was to determine if piglets develop an immune response to raw pea protein as calves do. It was also attempted to check the influence of the sow's diet on this response.

Materials and Methods

Animals. Two groups of three piglets were used in the experiment. The piglets of one group (*group I*) were weaned from sows fed a diet containing raw pea. Their live weight and age one week after arrival was 5.7 kg and 4 weeks. The other group (*group II*) were piglets of 7.5 kg live weight and 5 weeks of age, coming from sows fed a pea-free diet for at least 9 months before parturition. They were housed individually in metabolism cages.

Diets. One semi-purified diet was formulated (Table 1). The proteins were provided by the winter pea Frijaune and the pea protein concentrate obtained by air-classification of the same peas. The chemical composition is presented in Table 2.
All the piglets were fed restrictedly at 2.2 times their maintenance energy requirement (ARC, 1981) which was 4% of the body weight. The feed was offered twice daily (08.00 h, 16.00 h) as dry pellets (3 mm ∅; pelleting temperature 50-55°C). Water was freely available from nipple drinkers.

Table 1. Composition of the diet (in %).

Ingredients	
Raw peas[a]	25.0
Air-classified pea[a]	17.8
Maize starch	30.7
Dextrose	15.0
Sunflower oil	2.0
Cellulose	3.0
Vitamin/Mineral mix[a]	5.9

[a] For more detail, see Huisman et al. (1990).
In addition: DL-methionine .29%, L-threonine .06%, L-tryptophan .06%

Table 2. Calculated chemical composition (in %), analysed trypsin inhibitor activity (TIA, mg inhibited trypsin/g) and analysed lectin content (μg/g).

Items	
Net energy (kCal/kg)	2463
Crude protein (N x 6.25)	16.3
Crude fat	2.9
Crude fibre	5.0
Ash	6.2
Calcium	0.99
Available phosphorus	0.57
TIA	1.9
Lectin	1.9

Raw pea Frijaune : TIA = 2.1 mg t.i./g, Lectins = 3.6μg/g

Blood sampling. Blood samples were taken via tubes from the vena cava cranialis before the 16.00 h meal, on the day of the arrival of the piglets (week w_0) and then once a week at the same time during 7 weeks ($w_1 \ldots w_7$). After centrifugation, about 3 ml serum was separated and kept at - 20°C until analysis.

Antibodies. The indirect ELISA method has been applied to the serum to detect IgG antibodies against *Pisum sativum* legumin and against vicilin purified according to Guéguen et al. (1984). The method consists in coating ELISA microtiterplates (96 wels, NUNC brand) with legumin or vicilin solution, washing and incubating with the piglet serum. The piglet antibodies fixed on the pea antigens are detected using IgG raised against porcine antibodies and conjugated with peroxydase. The colorimetric reaction between the peroxydase and an added substrate is measured at 492 nm. The absorbance is proportional to the amount of antibodies raised by the piglets against pea legumin or vicilin.

No statistical analysis were performed as the number of animals was small.

Results and Discussion

Performances

Datas concerning the growth performances are presented in Table 3. They concern only two groups of three piglets without controls. Therefore they can not be generalized to piglets on a growth trial. The piglets of group I had a daily weight gain of 178 g *vs.* 207 g in group II, for a daily feed intake of 341 g and 437 g, respectively.

Table 3. Weight gain and feed conversion of piglets fed the raw pea diet during 7 weeks; piglets coming from a sow fed raw peas (group I) or from a sow not fed raw peas (group II).

Live weight ...	Initial	Final	Weight gain	Feed conversion
Group I	5.7 kg	14.5 kg	178 g/d	1.92
Group II	7.5 kg	17.7 kg	207 g/d	2.11

Circulatory antibodies against pea protein

The absorbance values of group I and group II measured individually have been pooled and the mean was calculated to get one value per pea protein and per week (cf. Figure 1). Serum antibody responses to pea legumin or pea vicilin were detected in all six piglets. An increase of up to 122% (legumin) and 134% (vicilin) was recorded within three weeks of commencement of feeding raw pea diet. The levels of antibodies decreased after week w_3 but increased again during week w_7. The response was similar for both globulin proteins.
In piglets of 4 to 8 weeks old, immune response has been shown to occur by means of egg white lysozyme inoculation (Hammerberg et al. 1989). Feeding a milk-substitute diet containing raw pea flour to calves led also to humoral antibody response against legumine and vicilin (Nunes do Prado et al. 1988). In their study the rate of increase of antibodies decreased four weeks after the start of the experimental diet.
The humoral response possibly indicates an increase of gut permeability to macromolecules or large polypeptides, meaning defects in the mucosal barrier. Increased gut permeability to macromolecules has been shown in veal calves fed raw pea flour, using ß-lactoglobulins, large molecules that normally do not go through the gutwall (Nunes do Prado et al. 1988). Toullec & Grongnet (1990) did not observed however the passage of ß-lactoglobulins in calves fed wheat or maize proteins, although a formation of antibodies against these dietary proteins was detected.
An enhancement of antigen uptake could result from toxic agents (Van Dijk et al. 1988). Lectins present in raw pea are maybe harmfull enough for the mucosal barrier to modify the permeability. Phaseolus bean lectins have been shown to stimulate the disruption of the microvilli in the jejunum of pigs (King et al. 1983).
Also a long-term presence of dietary proteins, which are not digested, may lead to an increased antigen uptake (Van Dijk et al. 1988). The protein of the actual diet had a low apparent ileal digestibility of 72% (Huisman et al. 1990).

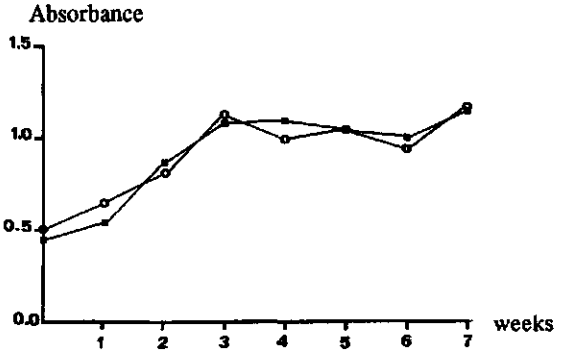

Fig. 1. Absorbance values measured at 492 nm for legumin (o) and vicilin (▪) in serum of piglets (pooled group I and II)

Influence of the mother's diet

The relation between the antibodies formation in piglets and their mother's diet can be evaluated on the starting point (w_0) and on the evolution of the response (w_1 to w_7).
The absorbance values measured for each group of piglets before they received any test feed (w_0) were low, and existed even when the piglets had not been in contact with pea proteins (group II) (Table 4). Prior to experimental feeding, low antibody titers were also found in veal calves against wheat or maize proteins (Toullec & Grongnet, 1990), or soya proteins (Barratt et al. 1978, Kilshaw and Sissons 1979, Guilloteau et al. 1986), or pea proteins (Nunes do Prado et al. 1988). A non-specific absorption of antibodies onto the antigen on the titerplates may be the reason.

When the piglets mother were fed peas (group I), the absorbance values at week w_0 were higher : + 77% for legumin, + 172% for vicilin. These animals had a prior experience of pea protein; either a passive transfer of antibodies from the sow via the colostrum or a co-eating of the sow diet by piglets.

Table 4. Influence of the sow's diet on the absorbance values at week w_0 when testing piglet serum for antibodies against pea legumin and pea vicilin.

Absorbance	Group I	Group II
Anti-legumin	.643 (n=1)	.364 (n=3)
Anti-vicilin	.682 (n=3)	.251 (n=3)

n : number of observations. Group I: piglets mother had peas. Group II: piglets mother had no peas.

The evolution of the immune response differed according to the groups. In group I, the response of the piglets was not only characterized by a higher starting point but also by a higher increase of antibodies formation against pea legumin than in group II (Figure 2). The increased difference was maintained from w_3 to w_7. For vicilin, the evolutions for group I and II were less clear (Figure 3).

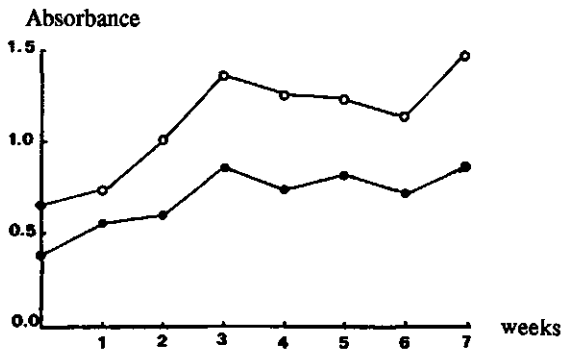

Fig. 2. Evolution of the absorbance values measured at 492 nm for **legumin** in serum of piglets of group I (o) and II (•) fed raw peas from week 0 until week 7.

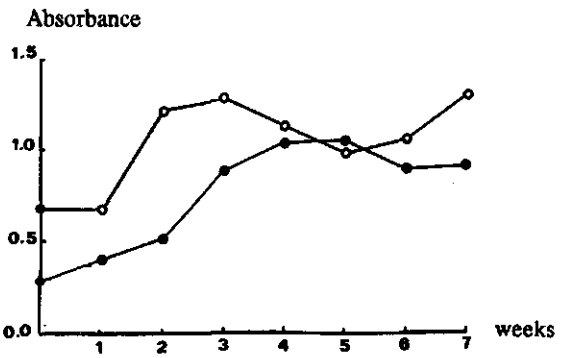

Fig. 3. Evolution of the absorbance values measured at 492 nm for **vicilin** in serum of piglets of group I (o) and II (•) fed raw peas from week 0 until week 7.

Conclusions

This small scale experiment revealed that antibodies against pea legumin and vicilin are being made by piglets fed raw peas. The intensity of the immune response is difficult to estimate as no negative control was used. No indication is available as for the gut permeability. The use of a large molecule (ß-lactoglobulin) as a marker or the analysis of immunoreactive legumin or lectin in the serum might have indicated whether the gut permeability was increased or not.
The observed immune reaction does not mean a food allergy; the piglets may have established an immunological tolerance (Van Dijk et al. 1988). If there had been a food allergy, gastrointestinal signs would have appeared; diarrhoea, weight loss, poor growth.
As for the mother's diet, the part of responsibility between maternal immunity or co-eating of the pea diet of the sow needs to be cleared up.

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MUCOSAL CATHEPSIN B AND D ACTIVITIES AND THE RELATION TO INTESTINAL MACROMOLECULAR ABSORPTION DURING POSTNATAL DEVELOPMENT IN PIGS

B.R. Weström, G.M. Ekström, N. Pantzar & B.W. Karlsson

Department of Animal Physiology, University of Lund, Helgonavägen 3B, S-223 62 Lund, Sweden.

Abstract

The aim of this study was to investigate the possible role of an alteration in the intracellular proteolytic capacity of the enterocytes in the mechanism of macromolecular transmission and gut closure. The intestinal mucosal uptake and transmission of fed macromolecules into blood was evaluated in piglets of several age groups and the activity of cathepsin B and D, the two major lysosomal proteinases, was measured in mucosal homogenates from the proximal and distal small intestine.

The results showed no significant differences in the activity of cathepsin B and D between newborn piglets and pigs 24 hours old, the latter having a confirmed postclosure status. Furthermore the proteolysis-resistant marker, FITC-dextran, showed the same transfer to the blood before and after closure as that of the protein markers. Thus, it is improbable that intestinal closure is due to an increase in mucosal intracellular degradation of internalized macromolecules.
Key words: Intestinal closure, Cathepsin B and D, Pig, Development

Introduction

The high transmission of milk-derived macromolecules from the small intestine into the blood of the neonatal pig ceases 18-36 h after birth at intestinal closure (Weström et al., 1984; Baintner, 1986). The present study was performed to investigate the possible role of an alteration in the intracellular proteolytic capacity of the enterocytes in the mechanism of gut closure (Ekström & Weström, 1990). The intestinal mucosal uptake and transmission of fed macromolecules into the blood was evaluated in piglets of several age groups and the activity of cathepsin B and D was measured in mucosal homogenates from the proximal and distal small intestine.

Materials and Methods

Nineteen Swedish Landrace piglets obtained from 5 litters were studied in the following age groups: newborn, unsuckled 0-3 h old; 24 h old; 6 days old and; 4-8 weeks old. The intestinal capacity to internalize and transmit macromolecules into the blood was evaluated by gavage feeding a marker solution containing bovine IgG (BIgG), bovine serum albumin (BSA), and FITC-conjugated dextran 70,000 (FITC-dextran). Four h later the pigs were anaesthetized, a blood sample was taken by puncture of the anterior vena cava and laparatomy was performed. Mucosal scrapings from the proximal and distal small intestine were frozen in liquid nitrogen and stored until analysis. For histological examination, 2 x 2 mm pieces were cut from the same sections.

Electroimmunoassay and radial immunodiffusion were used to determine the BSA and BIgG serum levels, and fluorescence spectrophotometry for the FITC-dextran levels, as described earlier (Weström et al., 1984).

Cathepsin B and D activities in the mucosal scrapings were determined according to Davies & Messer (1984) after homogenisation in ice-cold 0.15 M saline (1:1 w/v) and centrifugation at 16,000 x g for 30 minutes.

The tissue samples were fixed in ethanol/acetic acid (99/1) overnight at 4°C, paraffin embedded and cut in sections 5 µm thick, which were fixed in paraformaldehyde vapor (55-60°C) for 1.5 hour. Immuno-histological detection of the proteins was carried out in the sections after deparaffination, rehydration and incubation with primary antibody, rabbit anti-BSA or anti-BIgG and incubation with secondary antibody; TRITC-conjugated goat anti-rabbit-IgG. Evaluation of FITC-fluorescence was made after deparaffinating the sections.

Results

No significant differences in the mucosal activities of cathepsin B and D were found between the age groups (Table 1). In most cases, the activity present in the distal intestinal mucosa was higher than in that from the proximal section.

The transmission of all the markers to the blood 4 h after feeding were higher in the newborn pigs than in older, post-closure pigs, all of which only had minute amounts of the marker (Table 2). No apparent difference in the mucosal uptake of markers could be detected between the newborn, preclosure and postclosure pigs 24 h old. All three markers appeared together in a vesicular pattern in the enterocytes in the proximal portion and within a large vacuole in those located in the distal portion. In the 6 day-old pigs, uptake was only found in the distal small intestine, whereas no uptake could be found in the pigs 4-8 weeks of age.

Table 1. Mucosal activity of cathepsin B and D (mean±SD) in the proximal and distal small intestine during postnatal development in the pig. Specific activity for cathepsin B is given in nmoles p-nitroanilin/mg protein/min and for cathepsin D in µg degraded hemoglobulin/mg protein/min.

Age	n	Region	Cathepsin B	Cathepsin D
Newborn	7	Proximal	1.2 ± 0.4	156 ± 43
		Distal	2.6 ± 1.1	220 ± 43
24 hours	7	Proximal	0.6 ± 0.3	120 ± 20
		Distal	0.6 ± 0.3	97 ± 15
6 days	2	Proximal	0.8 ± 0.3	155 ± 106
		Distal	2.1 ± 1.3	280 ± 18
4-8 weeks	3	Proximal	1.1 ± 0.5	105 ± 21
		Distal	1.0 ± 0.2	280 ± 7

Discussion

Previous studies of neonatal animals have suggested that there is a relationship between the presence of mucosal lysosomal activity and the transfer of macromolecules across the intestine into the blood. This association has been studied in pigs (Brown & Moon, 1979); it was observed that the cathepsin B activity was higher in pigs 2 days old than in those 24 h old. However, the capacity for macromolecular transfer was not studied in those pigs nor was the cathepsin D activity.

Table 2. Mean blood serum levels (µg/ml) of markers 4 h after gavage feeding piglets of various ages.

Age	n	BSA	BIgG	FITC-dextran
Newborn	7	2000±460	2700±810	310±20
24 hours	7	109±100	87±109	9±9
6 days	2	-[1]	-	+[2]
4-8 weeks	3	-	-	+

[1]undetectable; [2]trace amounts

The results of this study showed no significant differences in the activity of the two major lysosomal proteinases, cathepsin B and D, between newborn piglets, and that of pigs 24 hours old, which had a confirmed postclosure status. The proteolysis-resistant marker, FITC-dextran, showed the same transfer to the blood before and after closure as that of the protein markers, further weakening the suggestion that intracellular proteolysis plays a major role in the mechanism of intestinal closure.

After closure, the internalized macromolecules will remain in the vacuoles to be degraded or to finally disappear from the mucosa due to cell shedding from the villus tip. The fetal-type enterocytes having a high endocytotic activity will be replaced by new adult-type cells having less endocytotic activity (Smith & Jarvis, 1977). This cell replacement proceeds in a proximal-distal direction, being completed in the proximal portion by the time the pig is 6 days of age, as observed in this study. Distally, there will still be uptake of macromolecules, but by the time the pigs are 4-8 weeks old this will not occur due to complete replacement of the cells.

In conclusion, proteolytic activity in the form of cathepsin B and D is present in the small intestinal epithelium of the young pig. Since the activity of these enzymes do not change at closure, it is improbable that closure is due to an increase in intracellular degradation of internalized macromolecules.

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ANIMAL SPECIES DIFFERENCES IN ANTINUTRITIONAL EFFECTS OF RAW PHASEOLUS VULGARIS BEANS AND PISUM SATIVUM: COMPARISON OF PIGLETS, RATS, CHICKENS AND MICE

J. Huisman[1], A.F.B. van der Poel[2] and A.C. Beynen[3]

[1]TNO-Institute of Animal Nutrition and Physiology (ILOB), P.O. Box 15, 6700 AA Wageningen, The Netherlands.

[2]Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands.

[3]Department of Laboratory Animal Science, State University, P.O. Box 80.166, 3508 TD Utrecht, The Netherlands.

Abstract

Experiments with young and older pigs, rats, chickens and mice were carried out to compare antinutritional effects of raw Phaseolus vulgaris beans and raw Pisum sativum. Weight gain in young pigs was much more negatively affected than in rats and chickens. The difference in sensitivity to ANFs in raw Phaseolus vulgaris between piglets, rats and chickens could not be explained by differences in physiological age. There was no reduction in weight gain of mice fed with raw Phaseolus beans.
Feeding raw Phaseolus beans and Pisum sativum caused an hypertrophy of the pancreas in rats and chickens. This was not observed in piglets.
The weight of the spleen was decreased in piglets with feeding raw Phaseolus beans, this was not found in rats and chickens.
Feeding Phaseolus beans and raw Pisum sativum caused a decreased weight of the thymus in piglets, but not in rats and chickens. The results demonstrate that digestive physiological and biological responses should be studied in the target animal. If this is the pig alternative small animal models should not be used.

Introduction

Many seeds contain substances which are referred to as antinutritional factors (ANFs) (Chubb, 1983; Friedman, 1986; Huisman, 1989; Huisman et al., 1990; Liener, 1980). This term is used because these factors can disturb metabolic processes and reduce the utilization of nutrients in the animal. The mode of action of ANFs has been studied mainly in rats, chickens and mice. Only a few studies have been carried out with pigs. In view of this, an important question is whether the results obtained in rats and other small laboratory animals can be extrapolated to the pig. Studies by Combs et al. (1967) and Yen et al. (1977) suggest that the rat and piglet respond differently to ANF in raw soya beans. Visitpanich et al. (1985) found different effects when chickpeas (Cicer arietinum) were fed. There is not enough information about the sensitivity of various animal species to ANFs in legume seeds.
Therefore experiments were carried out with rats, chickens and piglets fed Phaseolus vulgaris beans and peas (Pisum sativum) to compare the effects on weight gain and weight of organs.

In this paper the results (Huisman et al. 1990a, b, c) are summarized, expanded with recent results obtained with mice (Klaasen et al., 1991).

Materials and Methods

a. Experiments with piglets, rats and chickens.

In experiment 1, a comparison was made between rats and piglets fed raw and toasted Phaseolus vulgaris beans. Three diets were formulated, a control diet containing no beans and two test diets containing 20% raw beans or 20% toasted beans. A batch with a medium, high lectin content was selected. Part of these beans were steam heated for 40 minutes at 104°C and 19% moisture in the bean. The diets were balanced for total protein (about 18%), lysine, methionine + cystine, net energy, Ca and P. Each diet was fed to 15 rats and to 15 piglets. The piglets were of the crossbred Dutch Landrace x Dutch Yorkshire; the rats were Wistar animals. Weight gain in the piglets was measured in the period of 4 to 7 weeks of age and in the rats in the period of 5 to 8 weeks of age. At termination of the growth experiment, from each treatment 8 piglets and 8 rats were taken randomly for dissection and weighing different organs. With six rats and six piglets, not used for dissection, a faecal digestibility trial was carried out. Both the rats and piglets were fed restrictedly at a level of about 2.2 times maintenance requirement for energy. More details are described in Huisman et al. (1990a).

In experiment 2, the effect of inclusion of extra casein in the diet containing raw Phaseolus vulgaris was studied. The following treatments were involved: I, control diet containing casein and fishmeal as protein source; II, test diet containing 20% raw beans + extra casein; III, test diet containing 20% raw beans; IV, test diet containing 20% toasted beans.
The diets I, III, and IV were balanced for contents of digestible protein, amino acids, net energy, Ca and P.
The digestibility of protein in the toasted beans was found to be approximately 60%. This digestibility coefficient was used to balance the diets I, III and IV for digestible protein. In diet II, the same amount of casein and fishmeal was included as in the control diet. The digestibility of protein in the beans was assumed to be zero. For more details, see Huisman et al. (1990b). Each diet was fed to 12 piglets, 15 rats and 60 chickens. The piglets were of the crossbred Dutch Landrace x Dutch Yorkshire, the rats were Wistars and the chickens were Hybro birds. The weight gain was measured in piglets during 2 weeks in the period of 4 to 6 weeks of age, the rats during 3 weeks in the period of 5 to 8 weeks and the chickens during 3 weeks in the period of 1 to 4 weeks of age. The three animal species were fed on a restricted basis according to a scheme based on 2.2 times maintenance requirement for energy. At the end of the growth period from each treatment 7 piglets, 7 rats and 12 chickens were taken randomly and dissected for collection of various organs.

In experiment 3, the effect of inclusion of peas in the diet was compared in rats, chickens and piglets. Two diets were formulated: a control diet containing casein and fish meal as protein source and a test diet in which a part of the casein was replaced by 30% peas. Both diets were balanced for digestible protein, amino acids, net energy, Ca and P. Each diet was fed to 12 piglets of the crossbred Dutch Landrace x Dutch Yorkshire, 12 Wistar rats and 60 Hybro chickens. Weight gain of the piglets was measured in the period of 4 to 6 weeks of age, in the rats in the period of 5 to 8 weeks of age and in the chickens in the period of 1 to 4 weeks of age. At termination of the growth experiment, 7 piglets, 7 rats and 12 chickens were randomly chosen for dissection and collection of the organs. For more details, see Huisman et al. (1990c).

b. Experiment with mice

The sensitivity of mice to antinutritional factors in Phaseolus vulgaris beans was tested with raw and boiled beans. Three diets were formulated: control diet containing no beans, test diet containing 20% raw Phaseolus beans and a test diet containing 20% boiled Phaseolus beans. The mean lectin content in the raw beans was 43500 µg/g product and in the boiled beans 458 µg/g. Three experiments with mice were carried out. In each experiment each diet was fed to 10 mice. In experiment 1 six weeks old BALB/C mice were used and in experiments 2 and 3, seven weeks old Cpb:SE mice. After one week of adaptation, the diets were fed ad libitum during 30 days. More details are described in Klaasen et al. (1991).

Results and Discussion

The results of experiments 1, 2 and 3 with piglets, rats and chickens are summarized in Table 1. Detailed results can be found in Huisman et al. (1990a,b and c). These results clearly show that weight gain of piglets was distinctly more reduced than in rats and chickens with feeding raw Phaseolus vulgaris beans. The piglets lost weight. Inclusion of extra casein in a diet with raw beans (experiment 2) did not preclude the weight loss in piglets. In rats, there were hardly any negative effects on growth with extra casein. With peas, there was growth reduction in piglets but not in rats and chickens. For comparison of effects between animal species it is important that the design is monofactorial and that identical diets are used. The diets were therefore designed so that the only variable factor was the inclusion of beans. In our experiments, the feeding level for the three animal species was based on a similar low level related to metabolic weight. Especially for chickens this feeding level was very low. It may be possible that with higher feeding levels somewhat more negative effects in rats and chickens would be observed.

Table 1 Summary of the effects of ANFs in piglets, rats and chickens given diets containing raw Phaseolus beans and peas

Diet	Measurement	Effect		
		Piglet	Rat	Chicken
20% raw Phaseolus vulgaris	Weight gain	----	-/0	-/0
	Weight pancreas*	-/0	+	+
	Weight spleen*	-/--	0	0
	Weight of thymus*	-	0	0
	Protein digestibility	---	-	N.D.
30% Pisum sativum	Weight gain	-	0	0
	Weight pancreas*	0	+	+
	Weight of thymus*	-	0	0
	Weight spleen*	0	0	0

0 = no effect; + = increase; -/--/---/---- = decrease; N.D. = not determined; * = % of live weight.

In literature there are reports showing distinct negative effects on weight gain of rats fed raw Phaseolus beans. In these experiments, however, the diets mainly contained low levels of protein (often 10%) and high levels of raw beans (50% of more). In our experiments the protein levels of the diets were between 16 and 21%, and the inclusion level of the raw beans in the diets was more realistic, namely 20%. The results with mice show (Table 2) that these animals were not sensitive to feeding of raw Phaseolus beans under the conditions of the experiments. The results obtained with rats, mice and chickens show that under our experimental conditions there are no pronounced antinutritional effects with raw beans or raw peas. Piglets, however, show distinctly more antinutritional effects.

Table 2 Body weights (grams) of mice fed the experimental diets for 30 days.

Experiment	Initial body weight	Final body weights (± SD, n = 10)		
		Control	Raw beans	Boiled beans
1	14.1	15.6 (± 2.2)	15.4 (± 1.5)	17.0 (± 1.8)
2	24.3	25.7 (± 3.3)	27.4 (± 2.7)	26.9 (± 3.2)
3	26.1	27.8 (± 2.3)	27.2 (± 2.6)	27.2 (± 2.2)

For a full evaluation of the differential sensitivity of animal species, this should be studied at a similar physiological age. It is possible that a difference in physiological age is associated with a difference in sensitivity between rats and piglets. The piglets used in our studies were 4 to 7 weeks old and the rats were 5 to 8 weeks old. The physiological age of rats of a certain age will be different to that of pigs of the same age. Therefore, effects of the inclusion of raw beans on weight gain were also tested in pigs of other ages: 8 (Period P1), 12 (P2) and 16 weeks (P3), respectively (Table 3).

Table 3 Mean feed intake, weight gain and feed conversion ratio (kg feed per kg weight gain) with SD measured in pigs during 14 days

Treatment	Period P1		Period P2		Period P3	
	Mean	SD	Mean	SD	Mean	SD
			Feed intake (g/day)			
control diet	836a	13.4	1437a	6.2	2074a	20.3
raw bean diet	325b	37.7	621b	51.3	907b	127.1
			Weight gain (g/day)			
control diet	448a	32	626a	36	801a	53
raw bean diet	-65b	22	-154b	54	-128b	94

Means in the same column for the same parameter without a common letter differ significantly ($P<0.05$)

At the three ages, the pigs lost weight when fed raw common beans. These results suggest that the differences in response between young rats and piglets cannot be explained by a difference in physiological age.

In rats and chickens a hypertrophy of the pancreas was observed when the diets with raw beans or peas were fed; this was not found in piglets (Table 1). The hypertrophy in rats and chickens may be related with to presence of trypsin inhibitors in beans and peas (Liener and Kakade, 1980). The results with piglets are in agreement with the literature showing that there is no hypertrophy of the pancreas due to trypsin inhibitors in larger animals such as pigs (Liener and Kakade, 1980). The pancreas weight of the piglets fed the raw beans in experiment 1 was significantly lower compared with that of the piglets fed the control diet. There are indications that this is related with the low protein digestibility of the raw bean diet (Huisman et al., 1990a). With extra casein (experiment 2), pancreas weight of the piglets was not reduced. With raw beans the weight of the spleen of piglets was significantly reduced, but not in rats and chickens. With peas there were no effects on the weight of the spleen in the three animal species.

The weight of the thymus was significantly reduced in piglets when raw beans and peas were fed. This effect was not observed in rats and chickens.

The results summarized in this paper show that piglets are more sensitive to antinutritional factors in legume seeds than rats, mice and chickens. Moreover some biological responses in piglets are different from those observed in rats, mice and chickens. Therefore it is of importance to study digestive physiological and biological responses in the target animal, if this is the pig, and not in alternative small animal models.

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PLANT PHOSPHORUS RESPONSES TO SUPPLEMENTAL MICROBIAL PHYTASE IN THE DIET OF THE GROWING PIGS

Matti Näsi
Department of Animal Husbandry, University of Helsinki, Finland

Abstract

The effect of microbial phytase supplementation on phytin-phosphorus (P) availability for growing pigs on phytase deficient maize-soybean meal diets was measured in two digestibility and balance experiments. Apparent digestibility of P in diets without inorganic-P supplementation or with low addition (0.18 of total P) was significantly lower than in the control diets (0.16, 0.23 and 0.42; $P<0.01$), respectively. Phytase supplementation improved P digestibility ($P<0.01$) such that plant-P digestibility (0.40 and 0.28) rose to the same level as the control diet. P retention was also increased significantly by phytase supplementation. Calcium digestibility and retention were higher in phytase treated diets. Due to its effect on phytate-P availability, phytase treatment of feedstuffs allows a greater proportion of the pig's P requirement to be met by P of plant origin.

Introduction

In cereals and vegetable protein sources half to three quarters of the phosphorus (P) occurs organically bound with phytic acid in form of phytates. The availability of plant phytate-P is lower for pigs than that of non-phytate-P in plants or inorganic P of mineral sources and it shows variability among different plant feeds as reviewed by CROMWELL 1980, 1989, JONGBLOED 1987. In order for the pigs to utilize the phosphorus from phytate it must be hydrolysed by phytate-degrading enzymes to yield phosphates. Moreover, phytic acid binds strongly to other essential dietary minerals, such as calcium, zinc, magnesium, iron and copper, reducing their availability in the digestive tract of monogastrics (ZHU et al. 1990).

Although, phytase activity occurs in microbes and microbial phytases can be produced biotechnically, there are no reports of improved plant P utilization by pigs receiving phytase supplements (SHURSON et al. 1983 JONGBLOED 1987). However, in broilers phytase supplementation of diet (KIISKINEN and PIIRONEN 1990) and in chicks the addition of phytase producing organisms to the diet has resulted in a marked improvement in the utilization of phytate-P (NELSON et al. 1971). In pigs, similar attempts to supplement the diets with a dried yeast product have been unsuccessful (CROMWELL 1989).

The objective of this study was to evaluate the possibility of improve the the usage of phosphorus from plant ingredients by a phytase supplement produced by Aspergillus niger with a new technology. A maize-soybean meal pig ration containing low naturally occurring phytase activity was chosen as the test diet. Attention was also paid to the reduction of phosphorus content of in pig excrement in order to avoid the accumulation of phosphates in soils.

Materials and Methods

Two experients were conducted with six castrated growing pigs, in which

Table 1. Phosphorus excretion, apparent absorption and retention in pigs.

Exp.1

Treatments	1 Control	2 No phytase	3 Phytase	SEM	Statit. signif.
P intake	17.16a	9.30b	9.34a	0.107	***
P excret. in faeces	9.69a	7.12b	6.71b	0.197	***
P absorption, g/d	7.47a	2.18b	2.63b	0.207	***
P digestibility	0.434a	0.235b	0.284ab	0.0216	***
P excret. in urine	5.59a	0.38b	0.20b	0.317	**
P retained, g/d	2.23	1.79	2.43	0.380	NS
of intake	0.128	0.193	0.261	0.0312	NS
of absorption	0.312c	0.828ad	0.927bd	0.0315	**
g/kg metab.weight	0.07a	0.06a	0.09a	0.063	NS

Exp.2

Treatments	1 Control	2 No phytase	3 Phytase	SEM	Statist. signif.
P intake	17.19a	7.54b	7.65a	0.103	***
P excret. in faeces	10.07a	6.33b	4.59c	0.293	**
P absorption, g/d	7.11a	1.21b	3.06c	0.276	***
P digestibility	0.414a	0.162b	0.400a	0.0271	***
P excret. in urine	3.37a	0.16b	0.06b	0.094	***
P retained, g/d	3.72a	1.04b	3.00a	0.212	***
of intake	0.217a	0.139a	0.392b	0.0206	***
of absorption	0.523a	0.877b	0.979b	0.0283	***
g/kg metab.weight	0.12a	0.03b	0.10a	0.007	***

a - c: * P<0.05, ** P<0.01, *** P<0.001

the effect of added microbial phytase on the availability of plant phosphorus (P) was measured using balance assay.

The experimental diets were composed of maize (880 g/kg) and soybean meal (120 g/kg) and the daily allowances were 2500 - 2700 g. The control diets in both exps. were supplemented with dicalcium phosphate to give 6.5 g/kg total phosphorus in the ration. In exp. 1 diets 2 and 3 were supplemented with 3.3 g/kg of dicalcium phosphate to provide 0.50 of total P of the control diet mixture. In exp. 2 no inorganic P-supplementation was added to diets 2 and 3. In control diets, maize and soybean meal contributed 0.44 of the total supply of P and dicalcium phosphate 0.56. Calcium was added in all of the rations to provide diets with a content of 0.80 % total calcium. The trace elements and vitamins were supplemented according to Finnish recommendations (SALO et al. 1982) in all treatments.

Phytase product Finase-F (produced by Alko Ltd., Rajamäki, Finland) was used in both experiments. The level of supplementation in diet no 3 in

Exp.1 was 100 000 Pu/kg feed (1 Pu is the amount of enzyme that liberates 1 nmol of inorganic phosphorus from sodium-phytate in one minute, 37° C, pH 5.0) and 500 000 Pu/kg in Exp. 2, respectively. It was added to the ration in liquid form just before feeding. Finase-F preparation is produced by Aspergillus niger var. and the phytase is acid and heat resistant over broad pH and temperature ranges (pH 2.0 - 6.0 and up to 60°C).

The experiment had a 3 x 3 x 2 Latin square designs and the pigs had an average liveweight of 78 and 98 kg in exp. 1 and 2, respectively. Each period was comprised of 6 days of adjustment and 6 days of faeces and urine total collection. The chemical analyses of feeds and faeces were performed according to the official procedures. Phytic acid contents were analysed using the method of CAMIRE and CLYDESDALE (1982). Phosphorus was determined after dry ashing by the method of TAYSSKY and SHORR (1953) and minerals were measured on the diet ingredients, faeces and urine with a Varian Techtron AA1000 atomic-absorption spectrophotometer.

Results and Discussion

Average dry matter intake of the pigs was 79 g in Exp. 1 and 75 g in Exp. 2 per kg $W^{.75}$. Daily phosphorus intakes of pigs receiving the control diet were 17.2 g. Pigs on diets 2 and 3 in Exp. 1 consumed 9.3 g P/day, and in Exp. 2 consumed 7.5 g/day. Dicalcium phosphate contributed 0.56 of the total P supply in control diets and 0.18 of total P in diets 2 and 3 of Exp.1. No supplemental phosphorus was added in Exp. 2 on diets 2 and 3; all of the P supply was of plant origin (Table 1).

Organic matter digestibility was not different between the treatments in either experiment ($P>0.05$). Finase-F also contains some proteinase, amylase and pectinase activity, however, digestibility was not affected in this trial. The ash digestibility was improved significantly by phytase supplementation ($P<0.01$) in Exp.2. and showed similar tendency in Exp. 1. Nitrogen digestibility was significantly higher in Exp. 2. in pigs receiving diets without phosphorus supplement ($P<0.05$). However, there was no effect on N balance. A number of other investigations have pointed out that there is often strong binding between phytic acid and protein (ZHU 1990) but in this study phytase treatment did not result any improvement in protein digestibility.

The apparent digestibility of P on the control diet averaged 0.42 in the both trials and it was significantly higher ($P<0.05$, 0.01) than that of the diets without inorganic phosphorus supplementation (Exp. 2) or with low (0.18 contribution of total P) phosphate addition in Exp. 1 (Table 1). Phytase supplementation of the diet resulted a significant improvement in phosphorus digestibility ($P<0.01$) compared with that of the unsupplemented diet. Phytase improved plant-P digestibility such that it rose to the same level as the inorganic-P source supplemented diet (Exp. 2). Also in Exp. 1 phytase enhanced apparent digestibility of P by five percentage units ($P>0.05$). The apparent digestibility assay of the P in the total diet, however, poses some difficulties in the interpretation of the results, particularly when P supplies are different as in present study. Endogenous P secretions also complicate the measurement. An approximation of these daily P-losses in pig is 1.5 - 2.5 g/ 100 kg body weight (ARC 1981).

Similar low apparent P digestibilities as in present maize-soybean meal diets have been reported in cereals or cereal-based diets containing no

supplemental P (PIERCE et al. 1977, CALVERT et al. 1978, JONGBLOED 1987, OKSBJERG 1988). Phosphorus from phytate cannot be absorbed in its original form by pigs and so it must first be released by hydrolysis by phytate-degrading enzymes to yield inositol and phosphoric acid. Almost all vegetable feed ingredients posess phytase, although with different levels of activity (NELSON 1969). Maize has reported to have very low contents of natural phytase (CALVERT et al. 1978, POINTILLART et al. 1984, 1988, OKSBJERK 1988), which is why this diet was chosen to test microbial phytase supplementation eventhough maize is not used in pig feeding in Finland. In contrast to this the seed coats of wheat and barley have relatively high phytase activity. The optimum pH for plant phytase is about 5.0 (JONGBLOED 1987) therefore plant phytase probably does not survive the acid conditions of the stomach.

P digestibility in maize-based diets has been improved by treatments other than mirobial phytase supplementation. GUEGUEN and BAGHERI (1984) have shown that absorption coefficients of phosphorus in a maize SBM diet without added P was increased from 0.36 to 0.48 by introduction 200 g/kg wheat bran in the diet. Similarly, FOURDIN et al. (1988) reported that rye bran supplementation (200 g/kg) improved P retention 40 - 50 % in pigs on low P maize diets. In high-moisture maize the P is 3 to 4 times more available than in dried grain (BOYD et al. 1983). The improvements with those treatments have been similar or better than that of the present study of microbial phytase supplementation.

Besides being present in the feedstuffs, phytases are produced by the microbes in the intestine and also secreted by the intestinal tract of the pig and intestinal alkaline phosphatase can hydrolyse some phytate (POINTILLARD et al. 1984). However, it has been concluded that intestinal phytase does not seem to be of great significance for the hydrolysis of phytate (POINTILLART et al. 1984). In the large intestine with its rich mirobial flora phytate can be hydrolysed by the bacterial phytases. However, the pig's absorption of phosphorus in the large intestine is very limited and no P or only a limited amount of P was absorbed beyond the ileum fistula (JORGENSEN et al 1985).

The retention of phosphorus was 0.13 - 0.22 of P intake on the control diets and 0.14 - 0.19 on diets without inorganic P supplementation. This low availability is in accordance with existing literature. Microbial phytase supplementation improved retentions of phosphorus to the level of 0.26 - 0.39 and the difference was significant ($P<0.01$) compared with the untreated non P-supplemented diet. Retention of the absorbed P was very high (0.93 - 0.98) in diets supplemented with phytase and they were 0.10 higher relative to the untreated diets ($P<0.05$ in the first Exp). Some low-molecular weight myoinositol phosphates were probably absorbed but could not be utilized by the pig.

The retention of P in Exp. 1 was considerable lower compared to Exp. 2, but the reason for this remains uncertain. The effect of phytase supplementation was different between the two experiments due to the application level, which was five-fold in the second trial. The phosphorus availability in most inorganic phosphate supplements approaches 0.7 to 1.0 (CROMWELL 1989, JONGBLOED 1987, DEN HARTOG et al. 1988).

Calcium digestibility was improved by the phytase supplementation and retained Ca was significantly ($P<0.01$) higher compared to untreated diet. When the intake of Ca is adequate but that of P inadequate, the retention

of Ca falls (JONGBLOED 1987). Digestibility of magnesium also tended to be increased by the phytase treatment but retention was not affected. Iron supply was higher in the control diet due to the high content of iron in dicalciumphosphate. Fe digestibility was not different between diets but retention in the control diet was significantly higher. Copper and zinc digestibility and retention were higher in diets without phosphate addition but phytase supplementation did not affect any difference in utilization. Phytic acid readily forms complexes with several essential minerals such as calcium, iron, zinc and manganese and also protein impairing their utilization by the animal (NELSON 1971).

There are no previous reports of successful addition of microbial phytase to pig diets. However, many of the fungi, bacteria and yeasts are good sources of phytase. Attempts to improve phytate utilization in pigs by including a dried yeast product in the diet have been unsuccessful (CROMWELL and STAHLEY 1978). In contrast, several studies with chicks have demonstrated that the addition of phytase-producing organisms to the diet can result in a marked improvement in the utilization of phytate phosphorus (NELSON et al. 1971, CROMWELL 1980). SHURSON et al. (1983) was not able to improve phytate utilization in pigs by including a yeast phytase in the diet. It is possible that the additions of phytase have been insufficient or that the phytase used has not been resistant to low pH in stomach of the pig. In this study 100 000 Pu/kg was not sufficient because increasing phytase activity gave higher response in the Exp. 2. The Finase-F preparation is thermotolerant and acid-resistant to a pH value of 2. In poultry successful treatments has been also reported. NELSON et al. (1971) added phytase produced by a culture of Aspergillus ficium to the diet of chickens and found an increase in bone ash content compared to control. KIISKINEN and PIIROINEN (1990) showed improved plant-P digestibility in broilers after phytase supplementation.

In conclusion, the results of present study demonstrate that addition of microbial phytate degrading enzyme in maize-soybean meal-diet enhances the absorption of vegetable phosphorus and other essential minerals. More of the pigs' requirement for phosphorus could be met by P in maize and SBM if phosphorus could be converted to an available form. Phosphorus content in pig manure could be reduced followed the improved plant-P utilization with the present phytase treatment of the feed. Accumulation of P in cultivated fields is increased by using manures with high P content. Wheather the plant phosphate released by the enzymatic treatment is of economially feasible compared to other sources of phosphorus added to feed remains to be determined.

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ULTRASONIC AND ELECTROMYOGRAPHIC MEASUREMENTS OF THE EFFECTS OF FEED INTAKE ON DIGESTA FLOW AND GASTRO-INTESTINAL MOTILITY IN PIGS

J.W.Sissons and C.L.Jones

AFRC Institute for Grassland and Animal Production, Shinfield, Reading RG2 9AQ England

Abstract

Simultaneous recordings were made of gastric motility and digesta outflow in pigs fed either 1050g or 2250g of a wet 'grower' ration. Recordings showed that increasing feed intake raised the contractile activity of gastric muscle and the frequency of gushes of digesta entering the duodenum, but had little effect on gush size. They also indicated that these gastric muscles were quiescent and flow ceased during the initiation of a peristaltic wave on the duodenum. It is concluded that the stomach regulates the emptying of digesta by increasing the number of contractions which propagate through the antral-duodenal junction, instead of termination at the pylorus. Peristalsis in the duodenum also plays a regulatory role.

Introduction

Several factors are known to influence gastric emptying of digesta including central and local innervation of the stomach, contractile rhythms of the muscle wall, meal size, viscosity and composition. Few studies have examined the dynamics of gastric digesta outflow in the pig or gained understanding of the precise relation between contractile behaviour and digesta passage from the stomach.

Observations of digesta flow from gastric and duodenal cannulas suggest that meal size affects the rate of passage during the early phase of digestion (Low et al. 1985). But, it is uncertain whether the process of cannulation disturbs the normal physiology of the stomach. For example, adhesion of the gastric or gut wall to the body wall is likely to restrict the normal contractions of the smooth muscle. Also opening a cannula to collect digesta may introduce abnormal pressure changes within the lumen.

To overcome these objections, relatively non-invasive methods were used in the present work to study the effects of meal size on gastric function. Muscle contractions at the gastro-duodenal junction were measured by electromyography whilst simultanous recordings of digesta movement in the

proximal duodenum were made using an ultrasonic flow sensor.

Materials and Methods

Three male pigs (Large white x Landrace) weighing about 25 kg were surgically prepared with wire recording electrodes and an ultrasonic flow sensor (ID = 12mm) whilst maintaining oxygen/halothane anaesthesia. The electrodes were implanted on the serosal surface of the pyloric antrum, pyloric sphincter and proximal duodenum on either side of the flow sensor which was placed around the gut wall. The animals were fed a pelleted 'grower' ration containing (g/kg) 580.0, 200.0, 150.0, 50.0, 12.5, 5.0, 2.5 of barley, wheat, soyabean meal, fishmeal, mineral-vitamin pre-mix, di-calcium phosphate, and limestone respectively.

Two weeks after surgery the pigs were fed a test meal of either 300g or 600g of the 'grower' ration mixed with 750ml or 1500ml water respectively. During a 6h period after feeding, simultaneous recordings were made of myoelectric activity using a multi-channel polygraph (Grass Instruments model 7, Mass.) and of digesta flow from the sensor using an ultrasonic flow meter (Transonic Systems Inc. model T101, Ithaca). Variations in myoelectric potentials and ultrasonic flow were quantified using a summating integrator.

The effects of meal size on gastric function were compared from measurements of duration and intensity of myoelectric activity during successive post-prandial phases of antral motility, total digesta flow during each motility phase, rates of digesta outflow, gush size and frequency of gushes in the proximal duodenum. The significance of treatment differences was assessed using a paired t-test.

Results and Discussion

The pigs were kept for 4 weeks. They were at no time restricted in their movements in their pens. They gained, on average, 18 kg live weight. Post-mortum examination revealed no unusual tissue growth of the duodenum in the region of the sensor. This indicates that the surgical preparations had no adverse effects on digestive physiology or growth.

Motility of the gastro-duodenal junction

Observations of myoelectric activity showed that muscular contractions of the pyloric antrum occured in distinct phases. Successive phases were punctuated by the development of duodenal electrical activity of increased amplitude and regular rhythm (Fig.1). This activity is known to be linked with the onset of a peristaltic wave (Ruckebusch & Bueno, 1976). The duration of each motor phase recorded up to about 6h after feeding from the antral wall is shown in Table 1.

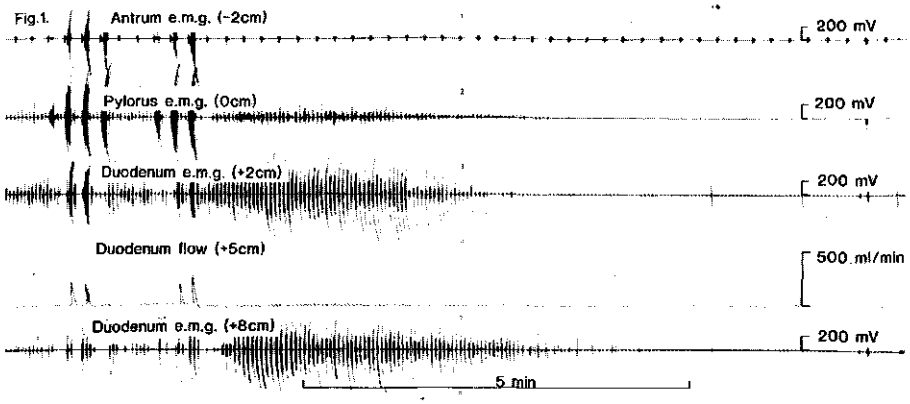

Figure 1: Electromyographic (e.m.g.) activity recorded from the antrum, pylorus and duodenum (values in parenthesis are distances from the pylorus) and digesta flow through the sensor. Duodenal recordings show the initiation and propogation of myoelectric activity linked with a peristaltic wave.

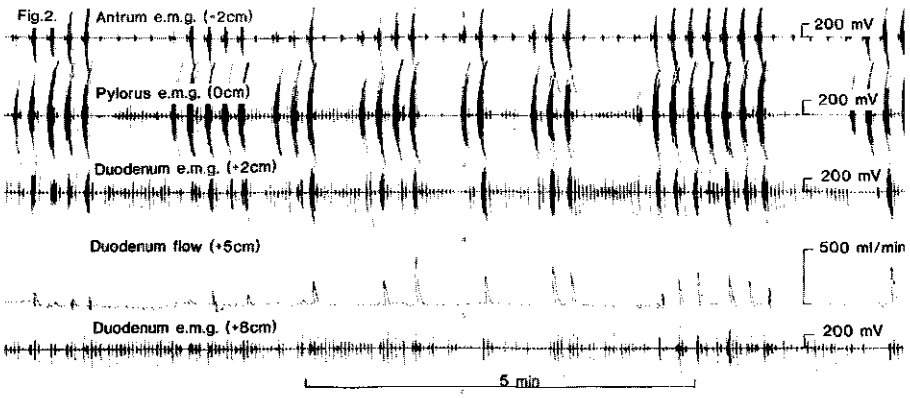

Figure 2: Electromyographic (e.m.g.) activity recorded from the antrum, pylorus and duodenum (values in parenthesis are distances from the pylorus) and digesta flow through the sensor. These recordings show a close relation between propagations of myoelectric activity from the pylorus through to the duodenum and gushes of digesta detected by the sensor.

Table 1. Duration of successive post-prandial phase of antral motility (h)

Feed intake (g):	Post-prandial phase of motility 1	2	3	4	5
1050	1.6	0.7	1.0	1.0	0.6
2250	1.8	0.6	0.9	1.0	1.2

Treatment differences were not significant (P>0.05)

The duration of the first post-prandial phase of muscular activity on the pyloric antrum was markedly longer than subsequent phases. This response to feeding has been reported elsewhere (Ruckebusch & Bueno, 1976).

Table 2. Integrated antral myoelectric activity (mV.s/h)

Feed intake (g):	Post-prandial phase of motility 1	2	3	4	5
1050	3.4	2.8	2.8	2.6	1.8
2250	3.6	5.2	5.0	5.8	5.6

Treatment differences for phases 3,4 and 5 were significant (P<0.05)

Although the amount of feed consummed did not affect the duration of each phase of antral motility, increasing the meal size did enhance the intensity of the myoelectric events during the second and subsequent motor phases (see Table 2). These alterations in electrical potentials are thought to involve tension receptors acting on the myenteric plexus and the vago-vagus reflex.

Passage of digesta

The amounts of digesta flowing from the stomach tended to increase with enlargement of meal size, in particular during the first phase of stomach motility following ingestion (see Table 3). Overall, during the 6h recording period 2100g and 2860g of digesta, on average, passed through the flow sensor after giving 1050g and 2250g of wet meal respectively.

Table 3. Total digesta entering the duodenum (ml)

Feed intake (g):	Post-prandial phase of motility 1	2	3	4	5
1050	828	361	415	314	184
2250	1041	483	446	485	403

Treatment differences for phases 3 and 4 were significant ($P<0.05$)

Results given in Table 4 show that the flow rate of digesta was greatest during the first two phases of gastric motility and then declined. An increase of feed intake led to a rise in digesta outflow during each of the 5 phases in the 6h recording period. These changes in flow were not, however, proportional to a doubling of the meal size.

Table 4. Digesta flow rate (ml/h)

Feed intake (g):	Post-prandial phase of motility 1	2	3	4	5
1050	530	590	430	330	300
2250	600	790	520	460	340

Treatment differences for phases 4 and 5 were significant ($P<0.05$)

The pattern of outflow of gastric digesta was to some extent related to variations in the size of gushes passing through the pylorus. These were found to increase during the first three phases of antral motility. Thereafter they decreased.

Table 5. Gush size (ml)

Feed intake (g):	Post-prandial phase of motility 1	2	3	4	5
1050	5.0	6.2	6.0	5.0	4.1
2250	5.1	6.4	6.7	5.3	3.9

Treatment differences were not significant ($P>0.05$)

It is noteable that the mean gush size did not correspond

to the intensity of antral motility. This may be explained from recordings which show that the occurance of a gush requires a contraction to pass from the antrum, through the pyloric sphincter and onward to the proximal duodenum (see Fig. 2). An exception to this aboral propagation was noted during the early phase of post-prandial activity when for a period of about 0.5h digesta movements oscillated, somewhat irratically, between forward and retrograde flow.

The frequency of gushes also varied with time after feeding, but unlike changes in gush size, the occurrence of the gushes was greatest during the first motor phase and then decreased progressively. Also the frequency of aboral digesta movements increased in most of the motor phases in response to a raised intake of feed.

Table 6. Frequency of gushes (no./h)

Feed intake (g):	Post-prandial phase of motility 1	2	3	4	5
1050	109	94	72	68	73
2250	120	93	93	90	88

Treatment differences for phases 3,4 and 5 were significant ($P<0.05$)

In conclusion, gastric emptying of digesta is not a continuous process, but occurs in discrete gushes. These gushes are propelled by muscle contractions which originate in the antrum. The frequency of the gushes is set by (1) the frequency of antral contractions and (2) whether a contraction is propagated through to the duodenum, rather than being terminated at the pylorus. The bulkiness of a meal provides a stimulus to this propagation and it appears to increase with meal size. Gastric outflow ceases during the development of a regular myoelectric rhythm on the proximal duodenum suggesting that innervation of the gastro-duodenal junction controls digesta emptying through a 'dual' mechanism.

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THE EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS ON WATER INTAKE AND WATER EXCRETION OF GROWING PIGS

Angelika Pfeiffer & H. Henkel

Institute of Animal Nutrition and Feed Science University of Kiel, Germany

Keywords: protein level, water intake, water excretion, N -excretion, ileal digestibilities, urea N, ammonia N

Abstract

A higher daily protein level results in an increased ($P < 0.05$) water consumption of the animals, which is corresponding with an increased volume of the daily urine excretion associated with higher concentrations of urea and ammonia N . Moreover a higher protein intake per day ends in a significantly higer flowrate of chyme at the terminal ileum as well as it contains significant higher ammonia- ($P < 0.01$) and urea- (N.S.) N concentrations.

Introduction

It is a well known fact that the daily water intake of an animal depends on a lot of factors. A very important parameter is the environmental temperature (MOUNT et al., 1971; AUMAITRE, 1965; STRAUB et al., 1976; NIENHABER & HAHN, 1984). An other critical point which should be taken into consideration is the feeding level (LEPKOVSKY et al., 1957; BARBER et al., 1963; BROOKS et al., 1984; YANG et al.,1984) associated with the liveweight and the age of the animals (AUMAITRE, 1965). The composition of the applied diet should be also considered (KRACHT et al., 1978/79; BROOKS et al.,1984)

Two experiments were conducted to study the effect of different protein levels on water intake and water excretion of growing pigs.

The objective of the first experiment was to examine the effect of diets with different crude protein contents on water excretion in growing pigs.

The aims of the second experiment were a) to study the apparent ileal digestibilities of nitrogen compounds in barley and soybean meal b) to estimate the effect of protein level on daily water intake and the excretion of water in the urine and faeces c) to measure the effect of protein level on the daily flowrates at the terminal ileum.

Materials and Methods

Pigs and their treatment

In both experiments the pigs were housed in metabolic crates. The environmental temperature was in a range of 18 to 20°C and relative humidity was 60 to 80% for the two studies. Water was supplied ad libitum and was measured per animal and day in gram.

Table 1. Initial weight and duration of measuring periods.

Treatment	Experiment 1 A	Experiment 1 B	Experiment 2 I	Experiment 2 II
protein level	low	high	low	high
No of animals	8	8	8	8
PERIOD 1				
initial weight (kg)	45,8	45,8	20,0	20,0
duration (days):				
water & N balance	5	5	7	7
ileal chyme collection			3	3
feed g/d (dry matter)	1742	1747	864	860
PERIOD 2				
initial weight (kg)	68,5	68,5	25,0	25,0
duration (days)				
water & N balance	5	5	7	7
ileal chyme collection			3	3
feed g/d (dry matter)	2089	2076	877	875

Experiment 1

Experiment 1 was conducted in the Institute of Animal Nutrition and Feed Science of the University of Kiel, Germany.
The experiment was carried out with 8 barrows from 20 to 100 kg liveweight in a cross over design (see table 1) respectively. The diets were based on barley and soybean meal and were calculated to be isoenergetic for metabolizable energy . The pigs were fed twice daily in two equal portions at the 3.1 maintenance level.

Treatment A: Barley + soybean meal with 18.5% crude protein (76% barley, 20% soybean meal) in period 1 and 14,4% crude protein (87% barley, 9% soybean meal in period 2 respectively; supplemented with synthetic amino acids (Ly, Met, Thr and Tryp)

Treatment B: Barley + soybean meal with 25% crude protein (62% barley 35% soybean meal in period 1 and 2 respectively.

After an adaptation period of 3 weeks urine was collected quantitatively over a period of 5 days. The urine was trapped in 10% H_2SO_4 to avoid any ammonia losses. Faeces were also collected during the same 5 day period by means of spot sampling and they were immediately stored at -25°C.
Acid- insoluble ash (3.5 N HCl) was used as a digestibility marker.

Experiment 2

Experiment 2 was undertaken in cooperation with the Department of Animal Nutrition of the Agricultural University of Wageningen and the Institute for Cereals, Flour and Bread TNO in Wageningen (The Netherlands).
For this trial 8 barrows from 20 to 30 kg liveweight were fed in a cross over design (as shown table 1) respectively. The pigs were fitted with post - valvular - T - cannulas at the terminal ileum (VAN LEEUWEN et al., 1988).
The diets were applied as a barley diet supplemented by minerals (diet I) and as a semisynthetic diet based on soybean meal and starch (diet II). The animals were fed twice daily at the 2.5 maintenance level.

Treatment I: barley diet with 12.54% crude protein

Treatment II: soybean - starch diet with 23.79% crude protein (45% soybean meal)

After an adaptation period of 7 days the ileal digesta were collected quantitatively in bottles which were cooled on ice and stored at -20°C. The chyme collection was practised for 3 days per period and for 24 hours per day with a 24 hours' interval between separate collecting days. Unrelated to these phases the daily water intake of each animal was measured during 7 days and urine and faeces were collected quantitatively for the same time.

Analytical procedures

Diets of both experiments were analyzed for dry matter, crude protein and ash. The data of the barley diet and the soybean starch diet were completed by the analysis of the pattern of amino acids.
A representative digesta-, urine- and feaces sample for each animal were analyzed for dry matter, crude protein and ash. The chyme data were supplemented by measuring the amino acids. The content of urea and ammonia - N in chyme and urine were also determined.
All analyses were carried out according to VDLUFA (NAUMANN et al.,1976) except urea - N SIGMA TEST COMBINATION No 535) and ammonia - N analyses (BRANDT, 1979 modified according to NAUMANN et al.1976).
Data were analyzed by analyses of variance with the LSD - Test, SAS PROGRAMME (GLM - Procedure) 1988.

Results and Discussion

Table 2 provides data on the daily nutrient intakes per animal and day.

Table 2. Water and nitrogen balances.+
g per animal and day

	Experiment 1			Experiment 2		
Treatment protein level	A low		B high	I low		II high
dry matter	1914	ns	1910	870	ns	867
crude protein	319	**	433	109	***	206
water intake	4318	*	5427	3286	**	4577
H2O excretion						
urine	1873	*	2893	1862	**	2999
faeces	1186	ns	1189	353	***	209
N intake	49.7	***	76.7	17.5	***	33.0
N excretion; urine	13,3	***	32,1			
Urea N excretion; urine	10,0	***	26,5			
NH3 N excretion; urine	0,6	**	0,8			
N excretion; faeces	12,9	ns	16.0	25.6	ns	25.8

+ least square means
* P < 0.05; ** P < 0.01; *** P < 0.001; N.S. P > 0.1

There are significant differences in contents except in dry matter (P> 0.1). Crude protein and amino acid intakes were different among treatments within each experiment (P< 0.05). As shown in table 2 the higher protein intake resulted in a clear difference in water intake of the pigs in experiments 1 and 2 (P< 0.05 and P< 0.01 resp.). This fact illustrates that there exists a powerful dependence of the animals' water requirement on the protein content in the diet (AUMAITRE, 1965; WAHLSTROM et al.,1970; BROOKS & CARPENTER, 1990). There are three responsible factors for the close correlation between protein and water consumption. Firstly protein metabolism in the urea - cycle leads to heat increment of the organism. This results in higher water requirement (CRAMPTON & LLOYD, 1954; LLOYD et al., 1978; BROOKS & CARPENTER, 1990). Secondly there is a

high water requirement of the organism for excreting urea and ammonia via the kidneys (LLOYD et al.,1978). Moreover, there are the osmotic properties of the amino acids in the small intestine requiring water.
Corresponding to the higher water intake there was a higher urine excretion ($P< 0.05$; $P< 0.01$ for experiments 1 and 2 resp.; see Table 2). As shown in Table 2 the higher nitrogen - intake resulted in an increased nitrogen - excretion ($P< 0.001$) by urine. This is associated with increased urinary urea ($P< 0.001$) and ammonia - N ($P< 0.01$) concentrations.
Urea - N - excretion constitutes the major part of daily nitrogen excretion.

After change from the high to the low protein level two animals in each experiment maintened their daily water consumption (see Figures 1 and 2).

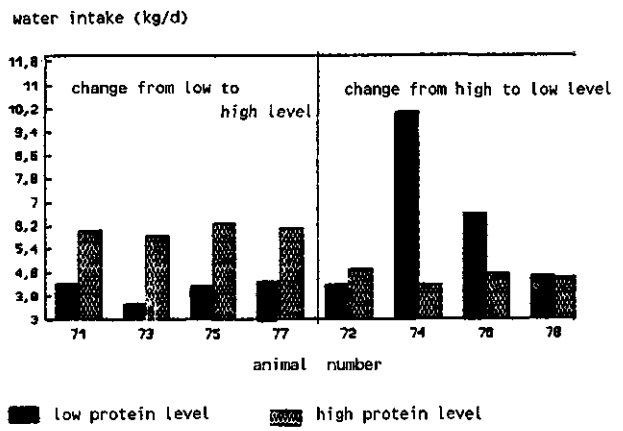

Figure 1. Reaction to the daily water intake due to applied protein amount (Experiment 1).

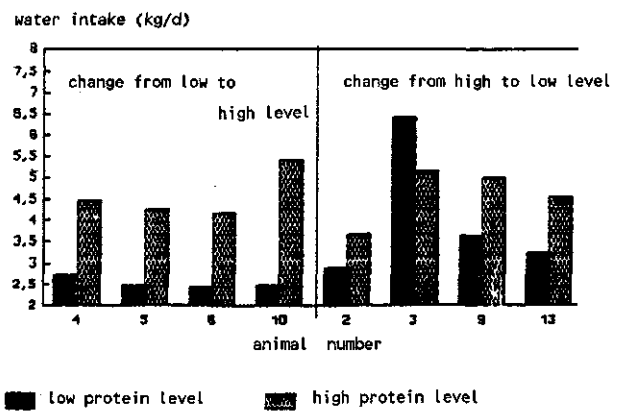

Figure 2. Reaction to the daily water intake due to applied protein amount (Experiment 2).

Table 3. Daily intake of nutrients as well as flowrate and disapparence at the terminal ileum.

Treatment protein level per animal / d	Experiment 2 Intake and flowrate I low		II high	% disapparence at the ileum I low	II high
g water intake	3286	**	4577		
g water; ileum	1640	**	2280	43,87	45,64
g dry matter intake	870	NS	867		
g dry matter; ileum	211	NS	208	75,75	76,01
g organic matter intake	826	NS	809		
g organic matter; ileum	186	NS	174	77,48	78,49
g crude protein intake	109	***	206		
g crude protein; ileum	26,4	**	41,7	75,78	79,76
breakdown products					
mg Urea N; ileum	347,0	NS	605,9		
mg Ammonia; ileum	240,9	**	430,8		

+ least square means
* P < 0.05; ** P < 0.01; *** P < 0.001; N.S. P > 0.1

This is not only valid for the time during the N-balance but also for the whole period (28 days). This reaction can be explained in the following way : It might be not necessary for an organism to lower the water intake when the protein level is decreasing. In contrast, it is essential to increase water consumption when the protein level is increasing. A water restriction causes a reduction in feed intake and in growth rate (CRAMPTON & LLOYD, 1954; KEANE et al., 1962).
Not only the daily flowrates of nutrients as given in Table 3 are significant higher for the soybean starch diet, but also the daily flowrate of breakdown products (Table 3) was increased, this was significant to ammonia - N ($P < 0.01$). But not for urea - N, there was a large but not significant increase ($P > 0.07$). Moreover there are remarkably differences in flowrates of water at the terminal ileum (see Table 3) between the diets . This is related to the higher water consumption of the animals consuming the diet with the higher protein level, and it is also resulting in higher water excretion.

Acknowledgments

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INFLUENCE OF ENVIRONMENTAL TEMPERATURE AND DIETARY PROTEIN LEVELS ON APPARENT DIGESTIBILITY OF PROTEIN AND AMINO ACIDS AND ENERGY BALANCE IN GROWING PIGS

Elias T. Fialho[1] and Tilford R. Cline[2]

1-Dept. Swine Nutr.,National Research Center Swine and Poultry, EMBRAPA/CNPSA.,Concordia, SC. , Brazil
2-Dept. Anim.Sci.,Purdue University,W.Lafayette.IN USA

Abstract

Thirty-six crossbred barrows weighing 23.1 $^{+}$ 0.4 kg were randomly assigned to one of six environmental-dietary protein combinations. The treatments were produced by a 2 x 3 factorial arrangement of treatments involving two environmental temperatures (25 ^{0}C and 35 ^{0}C) with three dietary protein levels (15%, 19% and 23% crude protein). The pigs were housed individually in steel metabolism cages in temperature controlled rooms. The data were analyzed as a split plot design with one complete replication. The data show that environmental temperature had a significant effect on ration intake and dry matter excreted, which were higher at 25 ^{0}C. The variables nitrogen retention blood urea nitrogen, apparent digestibility of protein, net protein utilization and biological value, were significantly ($P< 0.01$) higher for pigs housed at 25^{0}C than those at 35^{0}C. Increasing of protein level resulted in significant ($P< 0.01$) effects on those variables. The apparent digestibility of essential and nonessential amino acids were not influenced significantly ($P> 0.05$) by environmental temperature. For the diets with 15%, 19% and 23% of crude protein the average apparent digestibility of essential amino acids were 81.7, 86.7 and 88.5%, respectively. The digestible energy, metabolizable energy and ME/DE ratio values were higher at 25 ^{0}C than at 35 ^{0}C. Digestible energy was significant ($P> 0.01$) increased as protein level in the diet increased from 15% to 23%. According to the results of this experiment it is concluded that environmental temperature and dietary protein level have an influence on nitrogen retention, protein and energy utilization in growing pigs.

Keywords:swine,environmental temperature,fecal digestibility

1-Present address:Dept. Swine Nutr.,National Research Center Swine and Poultry, EMBRAPA/CNPSA., P.O Box 21, 89700 Concordia, SC. , Brazil

Introduction

Swine react to temperatures extreme by adjustments in feed intake and heat exchange with their environments.

Hot environmental conditions which reduce feed intake or cold conditions which stimulate feed intake may necessitate dietary changes in protein and energy if animal performance is to be maximized. If thermal stress affects protein requirements, the percentage of crude protein in the diet should be adjusted. There is some evidence that nitrogen retention is affected only by extreme environmental conditions, either in very cold (Close and Mount, 1976) or in very hot environmental conditions (Holmes, 1973).

Data in the literature indicate that digestibility of protein, amino acids and energy can be influenced by the environmental temperature in which the animals are exposed. Considering that the thermal stress affects nitrogen metabolism, protein levels in the diets may need to be adjusted. Further research on determination of the digestibility of nutrients in pigs under thermal stress conditions is required to improve our understanding of these subjects.

Metabolism trials were designed to quantify the interaction between environmental temperature and dietary protein level on energy and nitrogen balance as well as blood urea nitrogen, apparent digestibility of protein and amino acids in growing pigs.

Materials and Methods

This experiment involved a total of thirty-six crossbred (Landrace x Yorkshire), barrows, having an average initial weight of 23.1 $\pm$ 0.4 Kg were obtained from and housed at the Swine Research Facility at Purdue University.

The treatments were arranged as a 2 X 3 factorial involving two environmental temperatures (25^0 and 35^0C) with three diets of different protein content (15%, 19% and 23% crude protein) being conducted during two different phases (block 1 and 2) at each environmental temperature. There were, therefore, six environmental-dietary protein combinations. The metabolism assays involved a total of thirty six animals, with three animals being assigned to each of the different treatment combinations.

The pigs, were housed in environmentally controlled room in a total of twenty seven consecutive days.. The rooms, were maintained within 0.20 ^{0}C of the desired temperatures. During all metabolism assays, the pigs were kept individually in stainless steel metabolism cages (110 X 82 cm). The diets, were calculated to provide vitamin and mineral levels to meet or exceed the recommendations of the National Research Council (NRC, 1979). Nitrogen retention, apparent digestibility of dry matter, protein and amino acids, and digestible and metabolizable energy values were calculated from values based on the total collection of feces and urine.

The data were analyzed as a split plot design with one complete replication. The data were analyzed by using the General Linear Models Procedure of SAS (1985). Student-Newman-Keuls' Multiple range test was used to partition treatment means.

Table 1. Effect of environmental temperature and dietary protein levels on nitrogen retention, apparent digestibility of aminoacids, and metabolizable energy in growing pigs (Dry Matter Basis)

	Diet (% Crude Protein)									Temperature(oC)		
	15			19			23					
Item Temp.(oC)	25	35	$\bar{X}$ Diet	25	35	$\bar{X}$ Diet	25	35	$\bar{X}$ Diet	25	35	MSE[1]
Dry Matter Intake, g/day	1183	1073	1128^{a}	1188	1077	1132^{a}	1208	1073	1141^{a}	1193^{h}	1041^{i}	1235.8
App. dig. of Dry Matter, %	86.5	86.1	86.3^{b}	86.5	86.0	86.3^{b}	87.3	87.3^{b}	87.3^{a}	86.8^{h}	86.4^{h}	0.503
Nitrogen Intake, g/day	32.2	29.2	30.7^{c}	39.8	36.1	37.9^{b}	48.9	43.5	46.2^{a}	40.3^{h}	36.3^{i}	1.294
Nitrogen Retention, g/day	19.5^{c}	14.9^{d}	17.2	24.5^{b}	19.1^{c}	21.8	32.4	23.7^{b}	28.0	25.5^{h}	19.3^{i}	1.878
Blood Urea Nitrogen, mg/100ml	13.1	12.6	12.9^{c}	17.5	16.1	16.9^{b}	21.5	18.7	20.1^{a}	17.4^{h}	15.8^{i}	1.81
Apparent Dig. Protein, %	85.2	84.0	84.6	87.4	86.2	86.8^{b}	88.3	87.7	88.0^{a}	86.9^{h}	86.0^{i}	0.488
Lysine, %	79.0	77.2	78.1^{b}	86.7	85.3	86.0^{a}	87.8	87.9	87.8^{a}	84.5^{h}	83.5^{i}	4.97
Methionine, %	79.6	75.6	77.6^{b}	82.6	81.7	82.1^{ab}	84.5	84.1	84.3^{a}	82.2^{h}	80.5^{h}	7.98
Treonine, %	78.8	75.6	77.2^{b}	83.8	82.3	83.1^{a}	85.7	86.0	85.8^{a}	82.8^{h}	81.3^{h}	5.42
Digestible Energy, kcal/kg	3803	3788	3795^{c}	3892	3860	3877^{b}	3953	3916	3935^{a}	3883^{h}	3855^{i}	12933
Metabolizable Energy, kcal/kg	3658^{b}	3492^{a}	3575	3671^{b}	3552^{b}	3612	3676^{b}	3614^{b}	3645	3669^{h}	3553^{i}	1734

[1] Mean square error.

^{a,b,c,d} Means for dietary protein levels and ^{h,i} temperatures (lines) with different subscript letters are significantly different ($P > 0.01$).

Results and Discussion

The effects of environmental temperature and dietary protein, daily ration intake (RI), daily dry matter intake (DMI), daily dry matter excreted (DME), dry matter of feces (DMF), apparent digestibility of dry matter (ADDM), apparent digestibility of protein (ADP), daily nitrogen intake (NI), daily fecal nitrogen (FN), daily urinary nitrogen (UN), daily nitrogen retention (NR), blood urea nitrogen (BUN), net protein utilization (NPU), and biological value (BV) are studied. Therefore only the variables RI, DMI, ADDM, ADP, NI, NR, BUN, are presented in Table 1.

The data show that environmental temperature had a significant ($P< 0.01$) effect on RI, DMI and DME. All values were higher at 25^0C than at 35^0C. Dietary protein levels had no significant affect ($P> 0.05$) on these variables.

The variable FN was not significantly ($P> 0.05$) influenced by environmental temperature. However, NI, UN and NR were significantly ($P< 0.01$) influenced by environmental temperature. The increase of UN in the hot environment would suggest that protein catabolism was probably increased in the hot environmental temperature (35^0C). It is known that very high temperatures induce an increase in body protein catabolism resulting in increased urinary N excretion (Munro 1964). The increase of urinary nitrogen in a hot environment, was also reported in previous studies by Holmes (1973, 1974), who found that the hot temperature significantly increased urinary nitrogen with a corresponding reduction in nitrogen retention.

Nitrogen intake, FN, UN and NR showed a consistent protein response, being increased significantly ($P< 0.01$), as dietary protein level was increased from 15% to 23%. There was, however, a significant ($P< 0.01$) interaction between environmental temperature and dietary protein level for UN and NR, as shown in Table 1. The response was greater at $25\ ^0C$ than at $35\ ^0C$. The response also increased as dietary protein level increased in both variables analyzed. The interaction showed a greater increase with an increase in the dietary protein level at $25\ ^0C$ than $35\ ^0C$.

From nitrogen balance data (Table 1), it can be calculated that pigs fed low protein diet excreted less nitrogen in the urine than those fed the high protein diets.

Data from Table 1 show that BUN values were influenced by the environmental temperatures being greater ($P< 0.01$) for pigs housed at $25\ ^0C$ as compared to those kept at $35\ ^0C$. In addition, BUN values show a consistent protein response. The values increased significantly ($P< 0.01$), when protein levels were increased from 15% to 23% CP. High BUN levels seemed to be directly related with increased urinary N losses which also were increased by the increase in protein content in diets at both environmental temperatures. According to Reeds et al. (1981), the urinary nitrogen is derived from the catabolism of protein and amino acids by the animal, thus consumption of high protein results in increasing of urinary nitrogen and subsequent enhanced in BUN concentrations.

The data (Table 1) show that ADP as well NPU and BV were significantly ($P< 0.01$) higher for pigs housed at 25 ^{0}C than those at 35 ^{0}C.

The lower ADP at the higher temperature (35 ^{0}C) show that increasing the dietary protein levels also increased protein intake at both temperatures (25 ^{0} and 35 ^{0}C), but the difference in intake became smaller as the temperature increased (35 ^{0}C). This is due to changes in diet intake with the 35 ^{0}C temperature adversely affecting intake.

According to the literature it appears that the reduction in N retention, ADP, NPU and BV of diets in the hot environmental temperature (35 ^{0}C) is a real effect but the physiological reasons have not yet been established.

The data (Table 1) show that increasing levels of protein in the diet results in significant ($P< 0.01$) effects on ADP . Apparent digestibility of protein values increased significantly ($P< 0.01$) by the enhanced dietary protein levels, whereas NPU values were increased only from 15% to 19% CP. However, the BV values were not significantly ($P> 0.05$) affected by increased dietary protein level. The possible explanation for that may be is related to the relation between amino acids in terms of g/16g N that was maintained constant in all diets, which should have resulted in similar BV.

In this experiment the increase in nitrogen retention, BUN, ADP and NPU due to dietary protein levels and environmental temperatures were consistent. The positive effect of dietary protein levels on these variables show that the diets should be higher in protein content to compensate for the low feed intake in reaction to heat stress, in attempting to achieve an absolute crude protein intake. If feed intake is depressed by high ambient temperature it seems logical that there would be an improvement in protein utilization during thermal stress, by feeding animals with higher levels of dietary protein. Thus, when protein level is adjusted, growth rate during thermal stress should not be restricted due to a protein deficiency.

The influence of environmental temperature and dietary protein level on apparent digestibility of the essential amino acids are partially presented in Table 1.

In general, the apparent digestibility of essential (ADEAA) and non-essential (ADNEAA) amino acids, was greatest for pigs exposed to 25^{0}C environmental temperature compared to those in 35^{0}C, although the differences failed to reach significance ($P> 0.05$). Although, not significant, these findings are in general agreement with those reported by Wallis and Balnave (1984) who found, lower digestibility of most amino acids in broilers reared at higher temperatures.

The apparent digestibility of amino acids, values increased ($P< 0.01$) only as protein in the diet was increased from 15% to 19% CP; no further significant improvement ($P> 0.05$) were seen when protein levels were increased from 19% to 23% CP.

The average of apparent digestibilities of essential amino acids were 81.7; 86.7 and 88.5 for diets with 15% 19% and 23% ,respectively .

The effect of environmental temperature and dietary protein level on gross energy intake (GEI), daily fecal energy excreted (FE), daily urine energy excreted (UE), digestible energy (DE), metabolizable energy (ME) and digestible energy to metabolizable energy (ME/DE) ratio, part of these results are presented in Table 1.

In general all data related to energetic values were significantly higher ($P < 0.01$) for pigs housed at the thermoneutral environmental temperature ($25^{0}C$) in comparison with those above the upper critical temperature ($35\ ^{0}C$). This was expected, since at high ambient temperature the animal may have to activate heat loss mechanisms, which require additional metabolism that might result in lowering the efficiency of energy utilization.

Pigs exposed to hot environment conditions show reduced GEI, which is an attempt to lower the physiological burden of dissipating excess body heat in order to maintain body temperature. The DE and ME values were decreased as environmental temperatures were increased from 25^{0} to $35^{0}C$.

The data show that the GEI and FE values were not significantly influenced ($P > 0.05$) and DE values were significantly ($P < 0.01$) enhanced by increasing dietary protein levels from 15% to 23% CP. The improved DE (Table 1) efficiency was dependent on protein level in the diet. The higher protein supplied in the diet, the higher were the DE values. There was a significant interaction ($P < 0.01$) between environmental temperatures and dietary protein levels for the variables urine energy (UE), metabolizable energy (ME) and ME/DE ratio . The response was greater at $25^{0}C$ than at $35^{0}C$. The ME values were not significantly ($P > 0.05$) influenced by increasing dietary protein levels for pigs exposed to $25^{0}C$, however they increased significantly ($P < 0.01$) as protein levels increased from 15% to 19% CP at $35^{0}C$. The ME/DE values were decreased significantly ($P < 0.01$) as protein levels increased from 15% to 19% CP at $25^{0}C$, however they were not significantly ($P > 0.05$) influenced by increasing dietary protein levels for pigs exposed to $35^{0}C$.

The results of ME (Table 1) are apparently related to the heat production which must be dissipated under warm conditions (Verstegen et al. 1973; Noblet and Le Dividich, 1982). The reduced energy utilization during thermal stress was expected, since maintenance requirement for energy, according to those authors, increases during heat stress.

In general, diets with 19% crude protein fed to pigs housed at 25 ^{0}C seem to be adequate in terms of efficiency of protein and energy utilization. Based on the improvement of NR, ADP, NPU as dietary protein level increased in high environmental temperature of 35 ^{0}C (although less than in thermoneutral temperature), it seems that the protein concentration during heat stress should be increased in growing pigs kept in metabolism cages.

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COMPARISON BETWEEN THE ILEAL AND FECAL EXCRETION OF BILE ACIDS AND NEUTRAL STEROIDS IN PIGS

A.C. Beynen[1,2], J. Huisman[3] & C.E. West[2]

[1]Department of Laboratory Animal Science, State University, Utrecht, [2]Department of Human Nutrition, Agricultural University, Wageningen and [3]TNO-Institute for Animal Nutrition and Physiology (ILOB), Wageningen, The Netherlands

Abstract

Pigs fitted with re-entrant ileo-cecal cannulas fed purified diets containing either casein or soybean protein were used to study ileal and fecal excretion of neutral steroids and bile acids. Ileal and fecal excretion of neutral steroids was diminished in pigs fed casein, when compared with those fed soybean protein, suggesting that cholesterol absorption was stimulated by dietary casein. The output of bile acids in the feces of pigs fed casein was decreased, whereas the ileal output was not significantly affected. This could be attributed to increased uptake of bile acids from the cecum and/or colon, which may in part be related to the indirectly observed decreased formation of secondary bile acids.

Introduction

Pigs fed casein have been shown to excrete fewer bile acids and neutral steroids, that is cholesterol and its bacterial metabolites, with feces than their counterparts fed soybean protein (Kim et al. 1980). It is not yet known how, in molecular terms, dietary casein and its digestive products interact with neutral steroids and bile acids in the gastrointestinal tract. It also remains to be established where such interactions occur in the gastrointestinal tract. The present experiment using pigs fed diets containing either casein or soybean protein was conducted to address the question whether dietary casein interacts with intestinal bile acids and neutral steroids in the ileum or in the distal part of the digestive tract. The information thus obtained may also provide general insight into the digestive physiology of neutral steroids and bile acids. This study has been published in detail elsewhere (Beynen et al. 1990).

Materials and Methods

Twelve barrows derived from a cross between Dutch Landrace and Large White pigs were used. The animals were purchased from Coveco (Rheden, The Netherlands). On arrival they were 14 wk old and weighed about 35

kg. At 16 wk, 14 pigs were surgically fitted with a re-entrant ileo-cecal cannula as described (Beynen et al. 1986). The pigs were housed in metabolism cages (40x80x70 cm) constructed of stainless steel with wire mesh bases in a room with air conditioning (19 °C), 60-85% relative humidity and controlled lighting (0800-2000 h, light; 2000-0800 h, dimmed light). At the age of about 6 mo, all swine were transferred to the soybean protein diet for a base-line period of 10 d. Subsequently, on d 0 of the experiment, the barrows were randomly divided into two groups each of six animals. One group was fed the diet containing soybean-protein isolate and the other group was fed the casein diet; their body weights (means ± SE, n=6) were 56.2 ± 1.9 and 56.4 ± 2.0 kg. The composition of the purified diet containing soybean protein (or casein) was as follows (g): soybean isolate, 237.6; methionine, 2.4 (casein, 240); maize starch, 386 (377); soybean oil, 0 (13); coconut fat, 195 (192); cholesterol, 2 (2); cellulose, 101 (99.5); calcium carbonate, 0 (1.5); sodium chloride, 6 (8); and mineral, trace element and vitamin mixture, 74 (74). The composition of this mixture can be found elsewhere (Beynen et al. 1990).

Food was provided each day at 0900 h. The powdered diet was mixed with water in a 1:2 ratio in a slop. During the 10-d base-line period, each animal received 1200 g of air-dry feed/d. During the experimental period the amount of feed provided to each pig was equivalent to 2.4 times its maintenance requirement. The pigs consumed all their food within 10 min. On average, the pigs received 1218 g of air-dry feed per day. In addition, at 1600 h each day 2 L of water was supplied.

Feces were collected quantitatively from d 11 to d 18 and stored at -20 °C. Ileal chyme was collected quantitatively through the distal part of the cannula placed at the end of the small intestine from d 19 to d 21 and from d 24 to d 26. The loss of fluid with ileal chyme was compensated by infusing saline continuously (60 mL/h) into the cecum. Aliquots (10%) of chyme produced during both collection periods were pooled per corresponding hour and per animal thus producing a sample representative of 24-h chyme production. Chyme samples were frozen immediately (-20 °C) until analysis. Bile acids and neutral steroids in feces and chyme were measured by gas chromatography as described (Beynen et al. 1984).

Results

Body weights of the pigs fed soybean protein tended to be slightly higher than that of the animals fed casein. On d 18 and 28 body weights (means ± SE, n=6) were 68.0 ± 2.0 and 73.6 ± 1.1 versus 65.8 ± 1.4 and 72.3 ± 0.7 kg. The difference did not reach statistical significance.

Fecal excretion of total bile acids was about 35% lower in pigs fed casein than in those fed soybean protein (Table 1). This could be attributed partly to decreased excretion of lithocholic and isolithocholic acid, although the former did not reach statistical significance. Group mean excretion of total bile acids in ileal chyme was not significantly different in the two groups. Therefore, the calculated group mean net absorption of bile acids in the cecum plus colon was higher in pigs fed the casein diet than in the soybean-protein group (1718 ± 455 versus 1108 ± 243 μmol/d, means ± SEM, n=6).

Both ileal and fecal output of neutral steroids were lower in pigs fed casein than in pigs fed soybean protein, the decrease being 50% and 40%, respectively (Table 2). The lower output of ileal neutral steroids was caused mainly by lowered excretion of cholesterol, whereas in feces the content of coprostanol was diminished. The latter effect was

related to a significantly lower production of coprostanol in the cecum plus colon of casein-fed pigs (641 ± 84 versus 1961 ± 182 µmol/d, means ± SE, n=6). In the pigs fed soybean protein, significantly more cholesterol disappeared in the cecum plus colon than in the pigs fed casein (2415 ± 353 versus 753 ± 204 µmol/d, means ± SEM, n=6).

Table 1. Ileal and fecal output of bile acids in cannulated pigs fed purified diets containing either soy protein or casein.

	Output with ileal chyme		Output with feces	
Bile acid	Soybean protein	Casein	Soybean protein	Casein
			µmol/d	
Cholic acid	8± 4	11± 11	7± 1	4± 0[b]
Chenodeoxycholic acid	773± 79	850±170	58± 8	22± 6[b]
Deoxycholic acid	59± 5	5± 2[b]	14± 3	9± 3
Lithocholic acid	20± 2	12± 6	634±178	429± 62
Hyocholic acid	1725±152	1426± 97	n.d.	n.d.
Hyodeoxycholic acid	1231±105	1179±260	922±177	908±186
Ursodeoxycholic acid	n.d.	n.d.	9± 3	9± 1
Isochenodeoxycholic acid	n.d.	n.d.	14± 3	4± 1[b]
12-ketolithocholic acid	n.d.	n.d.	79± 15	0± 0[b]
Isolithocholic acid	n.d.	n.d.	275± 76	122± 20[a]
Unknown bile acids	n.d.	n.d.	797± 37	253± 52[b]
Total bile acids	3817±258	3483±460	2709±440	1765±278[a]

Results are expressed as mean ± SEM (n = 6). n.d. = not detectable. Significantly different from the soybean-protein group (one-sided t-test): [a], $p < 0.05$; [b], $p < 0.01$.

Cholesterol intakes (means ± SEM, n=6) of the pigs fed casein and soybean protein were 6003 ± 95 and 5776 ± 122 µmol/d. The lower fecal output of steroids in pigs fed casein resulted in a positive steroid balance (+2284 ± 401 µmol/d) (i.e., these animals appeared to retain steroids). In contrast, their counterparts fed soybean protein on average had a slightly negative steroid balance (-147 ± 477 µmol/d).

Discussion

In keeping with data reported by Kim et al. (1980), dietary casein significantly reduced the fecal excretion of bile acids and neutral steroids compared with soybean protein. Our data further suggest that absorption of bile acids in the cecum and/or colon is enhanced in swine fed casein, compared with those fed soybean protein (Table 1). This might be caused by less efficient formation of lithocholic and isolithocholic acid. These secondary bile acids are not as rapidly absorbed as others (Danielson & Sjövall 1975). Differences in the conversion of primary bile acids are possibly the result of differences in the bacterial flora of the gut of swine fed casein or soybean protein.

Table 2. Ileal and fecal output of neutral steroids in cannulated pigs fed purified diets containing either soybean protein or casein.

Neutral steroid	Output with ileal chyme		Output with feces	
	Soybean protein	Casein	Soybean protein	Casein
			µmol/d	
Cholesterol	3370±271	1666±287[b]	955±106	913±302
Cholestanol	33± 3	34± 2	77± 9	73± 4
Coprostanol	28± 2	17± 3[b]	1989±182	658± 85[b]
Epicoprostanol[1] + coprostanone	12± 3	2± 2[a]	164± 61	301±121
Total	3442±276	1720±290[b]	3213±177	1954±279[b]

Results are expressed as mean ± SEM (n = 6).
Significantly different from the soybean-protein group (one-sided t-test): [a], $p < 0.05$; [b], $p < 0.01$.
[1]No epicoprostanol was found in ileal chyme.

Dietary casein decreased the ileal output of cholesterol (Table 2). Theoretically, this could result from a decreased biliary cholesterol efflux in casein-fed swine. However, when Hagemeister et al. (1985) collected bile directly from the ductus choledochus, they did not observe a difference in cholesterol excretion between miniature pigs fed either casein or soybean protein in a cholesterol-enriched diet. This would imply that the difference in ileal output of cholesterol reflects a difference in efficiency of its ileal absorption rather than in the excretion in bile. This is consistent with data from studies with rabbits (Huff & Carroll 1980) showing that dietary casein stimulates cholesterol absorption when compared with soybean protein.

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SESSION 2

Endogenous losses during digestion in pigs

ENDOGENOUS NITROGEN LOSSES DURING DIGESTION IN PIGS

W.B. Souffrant

Research Centre of Animal Production Dummerstorf-Rostock
Division of Animal Nutrition "Oskar Kellner", Rostock, Germany

Abstract

For the evaluation of the capacity of feed proteins to supply amino acids it is essential to know the real protein digestibility and/or the amino acid absorption especially at the end of the ileum. For the assessment of these parameters it is necessary to measure the endogenous proportion of the nitrogen or amino acids in ileal chyme. The digestive tract and its exocrine glands secrete various amounts of nitrogen and amino acids in the lumen, part of which will be reabsorbed during the passage of the digesta through the gut. There are few methods to measure endogenous nitrogen (amino acids) in digesta at the terminal ileum of animals fed a protein containing diet. By means of the ^{15}N tracer technique this phenomenon can be studied. The results of such studies show that endogenous nitrogen losses also depend on the nature and level of the feed protein and on antinutritional factors present in the feed.

Keywords: pigs, endogenous nitrogen, endogenous amino acids, ileal digesta, nitrogen-free feeding, regression method, homoarginine method, isotope dilution method, recycling.

Introduction

Protein is an important component in the fulfilment of the nutritional requirements of monogastric domestic animals. In contrast with the other nutrients -fat and carbohydrates- which serve primarily as energy sources, the protein in feed is mainly incorporated as structure mass (Möllgard, 1955). For the synthesis of body protein amino acids are needed which must be ingested with the feed protein. A transition of feed protein in the organism can only occur in the form of amino acids and small peptides (Newey & Smith, 1964; Friedrich, 1982, 1989). The protein components of the feed must be degraded in the digestive tract to smaller molecules which can be absorbed and utilized by the animal.
The digestive processes in the alimentary canal are complex. They comprise secretion of digestive enzymes, enzymatic breakdown of feed components, microbial fermentation and finally absorption of the degradation products and utilization in the body cells.
According to Crane (1969) digestion and absorption cannot be considered as separate processes. Digestion and absorption in the digestive tract are closely related and affect each other,so they can be studied separately only for very specific questions (Young, 1970). Protein digestibility and amino acid absorption are calculated from the ingested (with the feed) and the excreted (with faeces or chyme) amounts of crude protein and amino acids. With this method all crude protein (or all amino acids) found in faeces or chyme are assumed to originate from the ingested feed. However, a definite part of the crude protein and amino acids present in the faeces and chyme is secreted into the digestive tract during the passage of the feed/chyme. This is why calculation of digestibility from crude protein (or amino acids) in the feed and in faeces or chyme yields the "apparent" digestibility and/or "apparent" absorption. Important parameters for the evaluation of the quality of feed proteins are the digestibility of the crude protein and the absorption of the amino acids. These parameters are used both for the calculation of adequate diet compositions and for the evaluation of

technological treatments.
A more precise estimation and evaluation of feed proteins with respect to their true digestibility and their capacity to supply amino acids is only possible when more knowledge is gained on the endogenous contribution of nitrogen and amino acids present in digesta or faeces.

Definition

In the past years, many research data on endogenous nitrogen and amino acids in chyme and faeces have been published. Irrespective of the methods used, the authors define endogenous nitrogen or amino acids as the proportion of nitrogen or amino acids in chyme or faeces that does not originate from the feed. The classical definition of endogenous nitrogen comes from Mitchell (1924).
According to his definition endogenous nitrogen is the nitrogen found in chyme or faeces when a nitrogen-free diet has been fed. This nitrogen is added to the digesta as enzymes, mucins, amides, amines, bacteria and mucosa cells during the passage of feed/chyme through the digestive tract. This definition excludes the endogenous nitrogen reabsorbed proximal to the site of chyme/faeces collection.

Sources of endogenous nitrogen and endogenous amino acids

In recent literature many experiments are described on assessment of endogenous nitrogen secretion in pigs. Some authors have focussed on the endogenous secretion by particular organs (e.g. Corring, 1975, 1979; Just et al., 1979; Tkacev, 1980; Schumann et al., 1983; Zebrowska et al., 1983, 1985) while others have studied the endogenous nitrogen content in several sections of the gastro-intestinal tract (Nasset and Ju, 1961; Nasset, 1964, 1972; Köhler et al., 1978; Souffrant et al., 1981; Taverner et al., 1981; Darcy et al., 1982, 1983).
The first addition of endogenous nitrogen takes place with the saliva. The nitrogen content of porcine saliva has been published only by Archipovec (1956) and Corring (1980). According to Archipovec (1956) the nitrogen content of saliva from pigs 107 - 110 days of age varies between 0.625 and 0.95 mg/ml, depending on feed intake. Per 100 g ingested feed, between 9.31 and 15.36 mg nitrogen is secreted with the saliva. For a pig of 30 - 40 kg live weight (daily feed intake 1.5 - 2.0 kg) this amounts to 162 - 216 mg of nitrogen secreted with the saliva every day.
Corring (1980) found with oesophagus-fistulated pigs of 45 kg live weight a maximum nitrogen secretion of about 400 mg/day. This amount accounts for approximately 0.8 % of the daily intake of nitrogen with the feed. Further data on saliva composition - amino acids and urea - were not given by these authors.
The gastric secretion in pigs has been studied using different methods with respect to pepsin and gastric juice secretion. Values given for gastric nitrogen secretion in swine show much variation due to difficulties in experimental techniques. The nitrogen sources in gastric juice are enzymes, mucins and cells sloughed off from the mucosa. Data on endogenous nitrogen secretion in the stomach are given by Tkacev (1980). In his experiments with pigs he found a daily endogenous nitrogen secretion in the stomach of about 9.6 g. Zebrowska et al. (1981) found endogenous nitrogen amounts of 2 g in mixed secretions of saliva and gastric juice. This amount accounted for ca 5% of the ingested nitrogen. In further experiments with ^{15}N-labelled wheat Zebrowska et al. (1982) measured ca 1.67 g of endogenous nitrogen in saliva and gastric juice over a period of 12 hours, corresponding with a daily amount of 3.3 g, being 8% of the daily amount of nitrogen ingested. Similar results - 20.4 g crude protein per day - were obtained by Simon et al. (1986) in experiments using the ^{14}C-leucine tracer technique with pigs weighing ca 35 kg and fed a cereal/milk powder diet. A description of the composition of gastric juice with respect to protein and amino acids cannot be found in the literature. Snary & Allen (1971) and Starkey et al. (1974) report data on the chemical composition of the cardial mucosa, indicating that threonine, glycine, glutamic acid, alanine

and serine are important constituents whereas histidine and aromatic amino acids are present in low amounts.

As compared to the sources of endogenous nitrogen secretion mentioned, data on pancreatic nitrogen secretion are abundant. Depending on the research method used the data on nitrogen secretion in the duodenum vary considerably. Values between 1 and 5.6 g/day have been reported (Corring & Jung, 1972; Corring, 1979; Tkacev, 1980; Gebhardt et al., 1981; Zebrowska et al., 1981; Partridge et al., 1982; Zebrowska, 1985; Ozimek et al., 1985; Souffrant et al., 1985), albeit most values are between 1 and 3 g nitrogen per day. Only the value reported by Tkacev (1980) (5.6 g/day) lies outside that range. This high nitrogen secretion value undoubtedly has to be ascribed to the fact that the author collected the pancreatic juice without reintroduction into the duodenum, thus causing hypersecretion of the pancreas due to a negative feedback mechanism. From the results of Corring & Jung (1972) it can be derived that 60 % of the nitrogen in pancreatic juice is protein bound. The remaining 40 % mainly consists of urea (Mosenthin, 1987). Schumann (1985) investigated the amino acid composition of pancreatic juice in response to different protein sources in the feed. The results of this study as well as data reported by Corring & Jung (1972) show high amounts of aspartic acid and glutamic acid, while methionine and histidine levels are low. To what extent the amino acid composition of pancreatic juice is affected by the composition of the feed remains unclear, although data from Schumann (1985) suggest a relationship. The feed composition certainly affects the daily amount of nitrogen secreted with pancreatic juice. The amount of nitrogen in pancreatic juice is lower in pigs fed a semi-synthetic diet than in pigs fed a simple diet. Both Zebrowska et al. (1981) and Partridge et al. (1982) found a difference of 0.4 g nitrogen secreted per day between the two types of diet. A higher dietary protein content resulted in an increase in protease activity in pancreatic juice but not in a significant change in the amount of nitrogen secreted (Corring, 1979). In contrast to this finding, different protein sources or differently treated proteins influence the nitrogen secretion by porcine pancreas (Schumann, 1985; Zebrowka et al., 1985).

The crude fat and crude fibre contents of the diet also affect the amount of nitrogen in the pancreatic juice of the pig. Ozimek & Sauer (1984) observed at a dietary fat content of 15 % an increase in the amount of nitrogen secreted of 0.4 mg as compared to a fat-free diet. An increase in dietary crude fibre content from 2.0 to 6.4% resulted in an increase of daily nitrogen secretion in pancreatic juice from 2.5 to 3.0 g (Zebrowska, 1985).

Bile secretion in the small intestine of pigs has been studied by Laplace & Ouaissi (1977), Sambrook (1978), Just et al. (1979) and Just (1982). Their results indicate that bile secretion is virtually independent of feed intake. The data on daily amounts of nitrogen secreted with the bile range from 1.8 - 1.9 g (Sambrook, 1981) to 2.8 - 3.0 g (Just, 1982) for pigs of 40 kg live weight. 75% of nitrogen in bile is α-amino nitrogen, 95% of which is glycine. One third of the remaining 25% of nitrogen is in the form of ammonium sulphate. The remaining two third has not yet been identified (Just, 1982).

The secretion of nitrogenous substances by the small intestinal mucosa consists of enzyme proteins and mucosa cells (Kidder & Manners, 1978; Aumaitre & Corring, 1978). Urea is also secreted by the gut mucosa (Rerat et al., 1979; Rerat & Buraczewska, 1985). Horszczaruk et al. (1974) studied the amount and composition of the intestinal secretion in swine using isolated gut loops. After feeding a diet containing 16% crude protein they calculated a daily amount of N in the small intestinal secretions of 10 to 12 g. After feeding a nitrogen-free diet only 8 to 10 g of N was secreted daily. The authors calculated these data assuming that the secretion rate in the isolated gut segment reflected the total small intestinal secretion. The results of Buraczewska (1979a), however, show that this assumption is not correct. She observed daily N secretion of 0.97 g/m in the proximal small intestine and of 0.48 g/m in the ileum. The total endogenous N secretion in the small intestine was found to be 14.4 g per 24 h (Buraczewska, 1979a). Low (1982), using data on protein synthesis and turnover given by Simon et al. (1978) and Edmunds et al. (1980), as well as the statement of McNurlan that 50% of the synthetized protein is secreted in the gut lumen, calculated that the daily N secretion of the small intestinal mucosa is 9.5 g. Research data on the characterization of the

composition of endogenous nitrogen secretions in the small intestine are presented by Buraczewska (1979b) who fitted pigs with two re-entrant cannulae in the small intestine to study the composition of the secretions in the isolated part of the gut. She found that 86-90% of the secreted N was soluble, of which 50 - 70% was α-amino N, which consisted of free amino acids (2/3) and protein (1/3). The remaining soluble N has not yet been identified, but will probably consist of amides, amino sugars and/or urea. The insoluble N in the gut secretions consists mainly of epithelial cells sloughed off from the brush border region of the small intestinal mucosa. Because of the short life cycle of these cells - McDougall (1966) reports turnover times of 50 to 80 hours - they form a relatively large proportion of the small intestinal secretion. Data on the amino acid content of small intestinal juice are reported by Horszczaruk et al. (1974) and Buraczewska (1979b). They found a high proportion of glutamic acid and aspartic acid and low proportions of methionine and histidine. The amino acid composition of the proteins in porcine pancreatic juice, bile and small intestinal secretions is given by Juste (1982) (Table 1).

Table 1. Amino acid composition of pancreatic juice, bile and small intestinal juice (Juste, 1982) (in % of total amino acids).

amino acid	pancreatic juice[1]	bile[2]	small intestinal juice[3]
Asp	11.3	0.43	10.01
Thr	5.9	0.25	6.14
Ser	7.8	0.26	5.96
Glu	10.2	1.10	14.38
Pro	5.4	0.25	5.77
Gly	5.9	94.86	5.51
Ala	5.8	--	5.63
Val	6.5	0.30	6.19
Ile	5.1	0.21	4.36
Leu	8.1	0.40	9.68
Tyr	5.5	0.19	3.26
Phe	4.7	0.24	4.96
Lys	5.5	0.34	8.39
His	2.5	0.23	2.61
Arg	5.1	0.27	6.50
Cys	3.4	0.56	--
Met	1.2	0.09	1.37

[1] Corring & Jung (1972)
the sum of the 17 amino acids was 74.3 g per 16 g N
[2] Juste, Corring & Calmes (unpublished)
the sum of the 16 amino acids was 65.45 g per 16 g N
[3] Buraczewska (1979)
the sum of the 16 amino acids was 50 - 70 g per 16 g N

From the data in Table 1 it appears that the proportion of amino acids in endogenous crude protein is 65 - 75%. The remaining 25 - 35% is largely accounted for predominantly by urea. According to Rerat & Buraczewska (1986) 4.6 - 6.1 g urea is secreted into the gut lumen during the first 8 hours after feed intake.

Studies on the direct measurement of endogenous N secretion in the large intestine have not been published to date. However, since the mucosa of the colon and caecum secretes mucins as well, endogenous N secretion in the large intestine can be expected to some extent. Low (1982) calculated - in the same manner as with small intestinal secretion - a daily endogenous N secretion in the large intestine of ca 3.0 g.
Another source of endogenous nitrogen and amino acids are microbes of the digestive tract. It is questionable whether their contribution can be regarded as endogenous; it is not secreted by the digestive tract or its exocrine glands, but it is always found in faeces and chyme. Microbes use for their metabolism nitrogen sources of both endogenous and exogenous origin. When the classical definition of "endogenous" is used microbial nitrogen should be included (Columbus, 1951).
The amount of bacterial nitrogen in faeces and ileal chyme has been assessed in studies with pigs fed protein-containing or protein-free diets. Mason et al. (1976) found that 50% of faecal nitrogen originated from bacteria. Mosenthin (1979) found, both with a simple and a complex diet, a proportion of 60% and Poppe (1982) a proportion of 50%. Meinl & Kreienbring (1985) found 67% of faecal nitrogen to be of bacterial origin in pigs on a cereal diet. With miniature pigs as a model Ahrens & Kaufmann (1985) demonstrated that bacterial fermentation in the large intestine, and hence the proportion of bacterial nitrogen in faeces, highly depends on the flow of carbohydrates into the large intestine. The amount of bacterial N can be much larger than the amount of endogenously secreted N. Ahrens & Kaufmann (1985) found with nitrogen-free feeding a bacterial N share of 90%.
Auclair (1986) summarized a vast amount of literature data on separate sources of endogenous N and their proportions of the entire endogenous N secretion in pigs (Table 2).

Table 2. Proportion of total and α-amino nitrogen of various endogenous N secretion sources in pigs (Auclair,1986).

source of endogenous N	total N		α-amino N*	
	g/24 h	%	g/24 h	%
salivary secretion	2.0 - 3.3	9 - 11	-	-
gastric secretion			-	-
pancreatic secretion	2.5 - 6.7	11 - 23	1.5 - 4.0	15 - 30
bile secretion	1.8 - 3.0	8 - 10	0.5 - 0.9	5 - 7
small intestinal secretion	14.4	65 - 49	7.8	59 - 77
sloughed cells	1.4 - 2.0	6 - 7	0.3 - 0.4	3 - 5
entire endogenous secretion	22.1 - 29.4	100	10.1 - 13.1	100

* soluble protein + free amino acids

From these data (Table 2) Auclair calculated the endogenous N secretion into the porcine digestive tract in relation to N intake (Table 3).

Table 3. Endogenous nitrogen secretion into the digetive tract of pigs, as a percentage of nitrogen intake (Auclair, 1986).

salivary secretion gastric secretion	5.0 - 8.0
pacreatic secretion	4.0 - 15.6
bile secretion	4.5 - 6.5
small intestinal secretion	22.0 - 26.5
sloughed cells	2.5 - 3.5
entire endogenous secretion	38.0 - 60.1

Depending on the literature consulted, data on the entire amount of N secreted into the digestive tract during the passage of digesta vary between 16 and 33 g. This endogenous N does not remain in the chyme up to exretion, but is reabsorbed, at least partly. The endogenous secretion of nitrogen and amino acids by the digestive tract and its glands is of special interest for research on nutitional physiology. The endogenous proportion of amino acids and nitrogen in different sections of the digestive tract, on the other hand, is of interest for the assessment of true protein digestibility and amino acid absorption.

Assessment of endogenous nitrogen in digesta and faeces

The classical method for the assessment of the endogenous pro-portion of nitrogen and amino acids is to measure nitrogen and amino acids in digesta and faeces of animals fed a N-free diet. Many authors have used this method in their studies (e.g., Holmes et al.; 1974, Pastuszewska et al., 1974; Sauer et al., 1977; Wünsche et al., 1979; Taverner et al., 1981; Darcy et al., 1982; Leibholz, 1982; Darcy-Vrillon & Laplace, 1984; Kies et al., 1986; De Lange et al., 1989). Wünsche et al. (1979) reviewed the literature on the endogenous nitrogen and amino acid content of ileal digesta from pigs. They compared their own results with data from 16 literature sources. Without making allowance for different diets, different methods for collecting ileal chyme and different live weights, they calculated the amounts of endogenous crude protein and amino acids (Table 4).

Table 4. Mean amounts of endogenous protein and endogenous amino acids in digesta from the terminal ileum and in faeces from pigs (Wünsche et al., 1987) (Values in mg/100 g dry matter intake).

	Ileal digesta			Faeces		
	mean	SD	n*	mean	SD	n*
Crude protein	1387	551	18	851	195	15
Asp	78.0	22.1	18	79.7	22.3	19
Thr	50.8	8.9	16	38.7	9.0	18
Ser	46.8	10.9	16	38.5	13.3	19
Glu	82.9	15.9	16	85.0	18.9	18
Pro	251.0	187.9	17	31.4	10.8	16
Gly	118.3	56.6	18	41.0	14.3	19
Ala	51.0	12.7	18	49.2	14.7	19
Val	40.0	9.9	16	44.7	13.3	19
Ile	24.3	8.0	16	37.0	10.2	19
Leu	49.6	17.8	18	52.5	15.7	19
Tyr	25.3	11.0	13	21.2	7.8	13
Phe	32.1	8.0	13	33.0	9.5	14
Lys	37.8	12.8	18	45.2	11.5	18
His	17.9	7.5	13	12.6	3.6	14
Arg	44.1	12.2	18	26.0	6.7	18
Met	10.4	4.4	17	18.8	6.4	18
Cys	20.5	10.3	10	15.2	5.2	11
Trp	18.3	3.3	4	8.0	1.0	3

* number of single values

From the literature data they calculated an endogenous N loss in ileal chyme and faeces of 2.2 and 1.4 g respectively per kg dry matter intake. The observed difference between chyme and faeces indicates that with N-free feeding approximately 0.8 g N is ab-sorbed per kg dry matter intake. According to Zebrowska et al. (1978) no amino acid absorption occurs after the end of the ileum. All N-containing compounds reaching the large intestine will be broken down by microbes and hardly be utilizable for the pig, because they are either used for synthesis of bacterial protein and free amino acids or absorbed as ammonia (Holmes et al., 1974; Mason et al., 1976; Sauer et al., 1977a,b). Part of the absorbed ammonia can re-enter the digestive tract in the form of urea (Rerat et al., 1979, 1985). It is not yet clear if amino acids syn-thetized in the large intestine can be reabsorbed, although results of Niiyama et al. (1978a,b) point in that direction.

The amino acid composition of small intestinal digesta from pigs fed N-free diets especially differs from the composition of faeces with regard to proline and glycine. According to Horowitz (1967), Corring & Jung (1972) and Low (1982) glycoproteins secreted with saliva and bile are rich in proline and glycine. The major part of these endogenous amino acids will be reabsorbed as bile acids during passage through the terminal ileum and proximal colon (Legrand-Defretin et al., 1986). Comparison of the amino acid composition of small intestinal

chyme and faeces shows a higher amount of methionine, isoleucine and lysine in faeces. This can be due to either proportional changes caused by absorption or metabolic action of microbes in the large intestine explaining the resemblance between the amino acid composition of faecal crude protein and that of bacterial protein (Poppe & Meier, 1983).
It is questionable if these broad mean values can be used to calculate true ileal and faecal protein digestibilities or amino acid absorption if it is taken into account that the amount of endogenous N at the terminal ileum can be affected by numerous factors. Bergner et al. (1975) and Taverner et al. (1981) found an increase in endogenous N losses with increasing dietary crude fibre content. De Lange et al. (1989) measured the endogenous nitrogen and amino acids at the terminal ileum and in faeces of pigs fed N- free diets with differing crude fibre and crude fat contents. Although they found high amounts of endogenous nitrogen (3.2 g N/kg dry matter intake) in their control group as compared to literature values, they did not find significantly increased N losses in ileal chyme neither with the fat-rich nor with the fibre-rich diet. However, the amount of endogenous N in faeces did increase when the fibrous diet was fed. A significant increase in endo- genous N in ileal chyme coinciding with a change in amino acid composition of the endogenous protein - especially with regard to glycine and proline - was found when a pectin-rich diet was fed. This was attributed to an increase in saliva and bile secretion.

An important disadvantage of the use of N-free feeding to assess endogenous nitrogen or amino acids in digesta or faeces is that it is not possible to investigate quantitatively or qualitatively relations between protein feeding and endogenous losses. Apart from the fact that N-free feeding is not a physiological condition and could evoke specific reactions in the animal body, it is not clear if data obtained with this method can be extrapolated towards different circumstances with regard to protein supplies.
De Lange et al. (1989b) recognized this point and studied endogenous nitrogen and amino acids in ileal chyme of pigs fed N-free diets and given intravenously an adequate mixture of amino acids to meet the needs of the animals. Under these conditions they observed a significant reduction in endogenous protein at the terminal ileum from 18.5 to 12.7 g per kg dry matter intake. As for the amino acids, they found a decrease only in proline from 3.6 to 0.6 g per kg dry matter intake. It is certainly not sensible to generalize these finding to oral protein supply. Nevertheless, the results of De Lange et al. indicate that a quantitative and qualitative relationship between protein supply and endogenous N losses at the terminal ileum can be expected.

Another method to measure endogenous N in digesta or faeces when protein- containing diets are fed is the regression method. Hereby, it is assumed that the proportion of endogenous N is constant when a protein-containing diet is fed, irrespective of the quantity of protein in the diet. With increasing protein levels in the feed the amount of N (or amino acids) in ileal chyme or faeces is measured and extrapolated to zero (linear regression) to find the endogenous N (or amino acid) secretion. This method has been used to assess true protein digestibility or amino acid absorption at the ileal or faecal level (Mitchell, 1964; Kristen, 1968; Poppe et al., 1969; Taverner et al., 1981; Moughan et al., 1987; Leibholz & Mollah, 1988).
Leibholz (1982) established with this method - by means of the slaughter technique to obtain ileal chyme - the endogenous nitrogen in ileal digesta of piglets. She calculated for a casein-containing diet approximately the same amount of ileal endogenous N as was found with N-free feeding (3.4 resp. 3.0 g N / kg dry matter intake). In a further trial Leibholz & Mollah (1988) investigated the endogenous nitrogen and amino acids in ileal chyme of piglets fitted with a T cannula at the terminal ileum and fed a protein- free diet or a diet based on cottonseed meal or milk. With the protein-free diet endogenous N at the terminal ileum amounted to 1.12 g / kg dry matter intake, while the milk- and cottonseed meal-based diets resulted in 0.94 and 0.89 g / kg dry matter intake, respectively. These differences were not significant, nor were the differences in amounts of endogenous essential amino acids. The differences in results between the two experiments were explained by differences in digesta collection methods and differences in live weight of the piglets (Leibholz & Mollah, 1988).

Because feed intake of the piglets was about ten times higher in the second study, it cannot be ruled out that endogenous secretion does not change in a linear manner with feed intake. Taverner et al. (1981) also used the regression method in their studies on ileal and faecal amino acid availability of cereal-based diets. They calculated endogenous N amounts in ileal chyme of 2.3 g per kg dry matter intake, but also noted that with diets balanced for crude fibre lower endogenous N levels were found than with N-free diets.

The regression method for calculating endogenous N and amino acids in chyme and faeces yields better results than the technique of N-free feeding: the former method makes it possible to assess the effects of protein quality and specific protein components (e.g., antinutritional factors). However, it seems not likely that a linear relation exists between feed intake and amounts of endogenous N or amino acids in digesta or faeces. Besides, an increase in the protein level in the feed is always connected with changes in the composition of the other crude nutrients and other feed ingredients, thus hampering the interpretation of results with regard to causes and effects.

Hagemeister & Erbersdobler (1985) published a new method based on labelling of feed protein, thus enabling discrimination between endogenous and exogenous protein in chyme. This method is known in the literature as the "homoarginine method" in accordance with the labelling procedure chosen. The feed protein is guanidinated so that the protein-bound amino acid lysine contained in the feed reacts with methyl-iso-urea thus forming homoarginine. Under natural conditions homoarginine is found only in traces in animal organisms. In contrast with other markers (^{15}N, ^{13}C, ^{14}C), it is not built in into endogenous protein, so intestinal scales and pancreatic protein are free of homoarginine. After absorption homoarginine is reconverted into lysine by arginase, an enzyme predominantly found in the liver; during that process urea is released. Provided that guanidinated protein and native protein are broken down enzymatically at similar rates in the digestive tract and that homoarginine is absorbed in a similar manner as the other protein-bound feed amino acids, the true ileal digestibility of feed proteins and - by comparison with the apparent ileal digestibility - the endogenous proportion of nitrogen in ileal chyme can be calculated on the basis of the amount of homoarginine that disappears during the chyme's passage up to the terminal ileum.

Hagemeister & Erbersdobler (1985, 1987) have investigated the ileal digestibility of casein and soya isolate in Göttinger miniature pigs. The homoarginine method enabled them to demonstrate that the true digestibility for both feed protein and endogenous protein was 99.5% and that more than 90% of the nitrogen found in the ileum was of an endogenous origin. For heat-damaged casein the authors found a reduced true ileal absorption of 93 - 97%. The homoarginine method can be applied only to feed proteins whose lysine is blocked, and hence cannot be labelled quantitatively, due to any heat treatment if there is no difference in ileal digestibility between labelled protein and protein inacessible to labelling. Hagemeister & Erbersdobler conclude that further experiments are needed to answer this question as well as to study the effect of alkaline treatments (as in the case of guanidination) on the digestibility of the protein involved. Schmitz (1988) , who has used the homoarginine method extensively, arrives at the conclusion that the method is applicable to absorption trials only if certain conditions are met. For example, lysine must be evenly distributed over the protein and be almost entirely convertible into homoarginine to achieve representative labelling of the protein. Except in case of heat damage, such an even distribution holds for most feed proteins (more than 80% for casein, soya isolate, lactoferrin and soya bean meal in Schmitz's experiments). The true ileal digestibility of non-representatively labelled proteins cannot be calculated from the elimination rate of homoarginine in the ileum.

Rutherford & Moughan (1990) have reported systematic experiments on guanidination of various feed proteins. They studied the effects of pH and protein level on the extent of guanidination of casein, gelatine and soya protein isolate. Lysine was not entirely converted into homoarginine in any of these proteins. Since guanidination was highest for gelatine (95%) these authors concluded that gelatine is an adequate protein source for the direct assessment of intestinal endogenous lysine secretion. Moughan & Rutherford (1990) found the extent of

guanidination of the test protein to be unrelated to the endogenous lysine flow in the ileal chyme of rats. There was no significant difference between partially and completely guanidinated dietary gelatine. Moughan & Rutherford applied the homoarginine method to trace endgenous lysine in ileal chyme of rats on diets varying in protein supply. They determined the endogenous lysine level of the ileal chyme upon a diet of either guanidinated gelatine, enzymatically produced casein hydrolysate, or a protein-free diet. The endogenous lysine flow did not differ significantly between both protein diets (487 and 522 μg respectively per g dry matter intake), but both the endogenous proportion of ileal lysine (238 μg/g dry matter intake) and the levels of other amino acids, except for glycine, were significantly lower for the N-free diet. The authors concluded that application of the traditional method with N-free diets leads to underestimates for endogenous nitrogen and amino acid values.

The homoarginine method is an appropriate method available to estimate the proportions of endogenous protein and amino acids in ileal chyme. The method of facilitates the assessment of the effects of protein content and quality on endogenous nitrogen and amino acid levels in the chyme at the terminal ileum alongside with the use of protein-free diets. Any drawbacks of the method can be removed by further systematic trials and its direct application to trials with farm animals.

One possibility to discriminate between the exogenous and endo-genous portions of nitrogen in chyme or faeces after feeding a protein diet is the use of tracers, in particular of the stable ^{15}N tracer. This method allows two approaches which are distinguished in that either the feed protein is labelled or the animal's nitrogen pool is labelled prior to the trials. Among those who have determined the endogenous portion of nitrogen in pig's chyme or faeces by means of ^{15}N-labelled feedstuffs are Krawielitzki & Smulikowska (1977), Köhler et al. (1978) and Souffrant et al. (1981). However, because ^{15}N-labelled feedstuffs are rather hard to obtain, ^{15}N labelling of the N pool of the experimental animals with various ^{15}N-labelled substances, administered either orally or intravenously, has been chosen as an alternative (Souffrant et al., 1981; Berger et al., 1984; Krawielitzki et al., 1984; de Lange et al. 1990). The endogenous N proportion in faeces or chyme could be calculated by means of isotope dilution. Irrespective of the approach for ^{15}N labelling chosen, the accuracy of endogenous N data depends on the question to what extent two prerequisites are met. First - a condition that can be assumend to be met indeed -, the absorption behaviour of labelled and unlabelled amino acids must be comparable. Second, the calculation can be reliable only if the endogenous N secretion does not undergo a significant change in extent of labelling by the absorbed nitrogen during the course of the experiment. The latter condition cannot always be considered realistic and implies that the calculated endogenous N values trend to be underestimates.

The use of the ^{15}N dilution technique for absorption trials has been reviewed by Souffrant et al. (1982) who also pointed at systematic problems. The applicability of the method is limited primarily by the cost of the ^{15}N-labelled substances. Particularly critical aspects on the ^{15}N dilution method are labelling of the animal's N pool, selection of the nitrogenous compound to be labelled,the method of application of the tracer substance, and selection of the substance whose labelling level is considered in the calculations to equal the labelling level of total endogenous nitrogen.

^{15}N-enriched ammonium salts and amino acids are suitable for labelling the N pool of experimental animals.Bergner et al. (1984), who supplemented the feed over a period of nine days with a mixture of [^{15}N] ammonium acetate and [^{15}N] ammonium chloride, achieved both in faeces and in blood of pigs ^{15}N labelling levels enabling an exact evaluation of the results. Herrmann et al. (1986) fed rats over a period of nine days on a ration enriched with ^{15}N-labelled amino acids (lysine or leucine). Krawielitzki et al. (1990) labelled the N pool of pigs with [^{15}N] ammonium sulphate as tracer substance, which was administered twice daily with the feed.

Souffrant et al. (1981) used [^{15}N] glycine and [^{15}N] leucine administered via continuous intravenous infusion to mark the N pool of pigs over a period of 8 - 9 days. Gebhardt et al. (1983) assessed the usefulness of various amino acids for labelling of the N pool by continuous

infusion. They recommend a mixture of ^{15}N-labelled amino acids, which is hardly feasible for economic reasons. In selecting the amino acids for use in an infusion one should take into account that they could be subject to transamination (so that the ^{15}N can be transmitted to other amino acids), that feed intake should not be impaired by the procedure, and that the method does not lead to imbalances. According to Gebhardt et al., glycine is not suitable as tracer amino acid for experiments aimed at establishing endogenous nitrogen, which is limited by the high selective secretion with the bile. ^{15}N-labelled leucine can successfully be used to label the N pool of pigs; a daily dose of 40 mg [^{15}N]-L-leucine per kg live weight has been found to be suitable for continuous intravenous infusion over 8 - 9 days.

The aim of oral adminstration or infusion of ^{15}N is to achieve a ^{15}N steady state in the N pool of experimental animals. Such a state is achieved only when the level of markers in faeces, urine and blood hardly increases any longer (Krawielitzki & Bock 1976) so that exogenous and endogenous N can be distinguished accurately in faeces or chyme. Since the extent of labelling of the N pool of experimental animals cannot be measured directly one must rely on a reference substance assumed to approximate the ^{15}N affinity of endogenous nitrogen. Both urine and blood - also because of their ease of sampling - are obvious matrices. However, because ^{15}N labelling of urine is influenced by many factors, such as the intermediary utilization of feed proteins, it yields unrealistic values when used as a reference for the calculation of endogenous nitrogen in chyme or faeces (Herrmann et al. 1986). For various reasons, the TCE- soluble blood plasma fraction appears to be a more favourable reference for measuring ^{15}N labelling. That fraction reflects the pool of amino acids required for protein synthesis, and hence for the formation of endogenous proteins, irrespective of whether these derive from intermediary protein conversion or have been supplied by absorption of this fraction. Also, the urea contained in the TCE-soluble fraction is an important component of endogenous N secretion (Rerat et al. 1979). Departing from these arguments, Herrmann et al. (1986) and Souffrant et al. (1981, 1986) have used the TCE-soluble fraction of blood plasma as a reference for the assessment of ^{15}N labelling of chyme.

The results obtained so far with the ^{15}N dilution method permit to conclude that the method can successfully be applied to the calculation of endogenous nitrogen in chyme or faeces. In combination with other experimental procedures, in particular experimental animal surgery, the method not only facilitates measurement of endogenous nitrogen in chyme or faeces, but also allows statements to be made as to recycling of endogenous nitrogen (Souffrant et al. 1986; Krawielitzki et al. 1990).

Recently, the proportion of endogenous amino acids in chyme or faeces could not be established directly with the ^{15}N dilution method. Published data on true precaecal amino acid absorption were calculated by attributing the amino acid composition analysed in the ileal chyme upon a N-free diet to the amount of endogenous protein found (Souffrant 1986; de Lange et al. 1990). Although the direct assessment of endogenous amino acids in chyme and faeces with the ^{15}N tracer technique appears to be possible in principle, many problems remain to be solved.

Endogenous nitrogen in chyme and faeces in the terminal ileum of pigs fed on various protein carriers

There is a vast body of literature on the proportion of endogenous nitrogen in the ileal chyme of pigs on a protein diet determined by means of the ^{15}N dilution method (Köhler et al. 1978; Souffrant et al. 1981a, b; Krawielitzki et al. 1990; de Lange et al. 1990; Huisman 1990). Köhler et al. (1978) investigated the proportion of endogenous nitrogen in pigs with a live weight of 50 kg by means of re-entrant cannulae in the proximal and terminal ileum. The only dietary protein source in their trials was ^{15}N-labelled dry curd. In the ileal chyme they found 2.9 g of endogenous nitrogen within 24 hours after feeding. Centrifugation and selective separation techniques enabled them to demonstrate that 30% of the endogenous nitrogen in the chyme at the terminal ileum was in the form of protein, 14% in the form of

peptides, and 29% in the form of free amino acids (Table 5). In the proximal part of the small intestine they found 12.5 g endogenous nitrogen within 24 hours.

Table 5. Contribution of nitrogenous compounds to endogenous nitrogen in the chyme of the proximal and terminal ileum of pigs, in g N per 24 hours. (After Köhler et al. 1978).

	Total N	Protein	Peptides	Free amino acids
Proximal ileum	12.5	3.1	2.6	3.5
Terminal ileum	2.9	0.9	0.4	0.8

Souffrant et al. (1981a) feeding the same protein diet (dry curd) to pigs varying in live weight between 15 and 27 kg found in the chyme of the terminal ileum 1.1 g N per day on the average, 60% of which was in a TCE-soluble (and hence absorbable) form. When live weight is taken into account the amount of endogenous nitrogen derived from ^{15}N-labelled dry curd was in fair agreement with Köhler et al.'s data (58.0 vs. 56.6 g N per kg live weight). Krawielitzki et al. (1990) investigated the endogenous proportion of nitrogen in the chyme of the terminal ileum of pigs fed soybean meal. Oral doses of [^{15}N] ammonium sulphate were used to label the animal's N pool. The amount of endogenous nitrogen in the ileal chyme recovered by means of the isotope dilution method was 3.8 g per day, or 1.7 g per 100 g crude protein intake.

Various research groups have recently investigated endogenous nitrogen in the ileal chyme of growing pigs by using prolonged infusion of [^{15}N] leucine and a wide variety of protein compounds (Souffrant et al. 1981b; de Lange et al. 1990; Huisman 1990). Their results are summarized in Table 6.

Table 6. Endogenous nitrogen in the ileal chyme of pigs fed various protein feeds.

Protein source	Endogenous nitrogen recovered		
	g/day	g/100 g protein intake	g/kg dry matter intake
Soybean meal[1]	5.8	2.2	4.1
Rapeseed meal[1]	7.3	2.9	4.9
Wheat[1]	5.8	3.1	4.4
Barley[1]	5.6	4.0	4.4
Peas cv. Finale[2]	1.6	2.7	4.9
Peas cv. Frijaune[2]	1.8	3.0	5.4
Phaseolus beans[2]	5.1	10.8	16.1
Casein[3]	2.4	2.0	4.1
Field bean protein isolate[3]	5.6	3.4	5.3
Soybean protein isolate[4]	2.0	3.3	5.7
Soybean meal[4]	1.4	2.2	4.2
Field bean cv. Blandine[4]	1.6	2.5	4.8
Field bean cv. Alfred[4]	1.8	2.7	5.2
Fish meal[4]	1.6	2.6	4.6
Dried skim milk[4]	0.7	1.3	2.2

[1] De Lange et al. (1990); pigs' average live weight 40 kg.
[2] Huisman (1990); pigs' average live weight 10 kg.
[3] Souffrant et al. (1981b); pigs' average live weight 40 kg.
[4] Heinz et al. (19..); pigs' average live weight 10 kg.

The absolute amounts of endogenous N in the chyme of the terminal ileum found in these studies varied between 0.7 and 7.3 g. These values are not directly comparable due to the variation both in live weight and in dose levels of protein and dry matter. Comparison of the various values is facilitated when the intake of dry matter or protein is accounted for. However, since the amount of endogenous nitrogen present in the ileal chyme could not, or not exactly, be determined on the basis of protein feeding, all values found so far were related to intake of dry matter. Many groups have found by regression analysis highly significant associations between endogenous nitrogen in the ileal chyme and dry matter intake. For assessment of the association between protein source and endogenous nitrogen level in the ileal chyme the relation with protein intake looks more promising. The lowest value of endogenous nitrogen was found for dried skim milk as the protein source (1.3 g/100 g crude protein intake). The highest value (10.8 g/100 g) was found for Phaseolus beans. These extreme values are directly comparable and reflect the specific reaction of piglets to these protein sources which are extremely divergent with regard to protein quality and content of antinutritional factors.

The precision of the ^{15}N dilution method for the determination of the endogenous proportion of nitrogen in ileal chyme or the sensitivity of the piglets with regard to the reaction of their digestive tract to the intake of feedstuffs varying in content of antinutritional factors can be assessed on the basis of the results obtained with both varieties of peas and field beans. Pea cv. Frijaune has a considerably higher trypsin inhibitor content than cv. Finale, and the tannin content of field bean cv. Alfred ist higher than that of cv. Blandine. The results obtained with soybean meal, however, show that the relative proportion of endogenous

nitrogen is equally high in the chyme at the terminal ileum of pigs varying in live weight.

It can be concluded from Table 6 that the content of endogenous nitrogen in ileal chyme of pigs ist not an invariable, but rather is affected by many factors. Besides the composition of crude nutrients in the diet, protein quality, the content of antinutritional factors, and the animals' age or live weight are important factors affecting the content of endogenous nitrogen in the chyme of the terminal ileum. Another factor capable of affecting the amount of endogenous nitrogen in ileal chyme is re-absorption of endogenous nitrogen.

Reabsorption of endogenously secreted nitrogen in the pig's digestive tract

There is a vast body of literature on endogenous secretion of nitrogen and amino acids in the digestive tract and on the endogenous proportion of nitrogen in the chyme in various sections of the gastro-intestinal tract of pigs. In contrast, studies on reabsorption of endogenous nitrogen are scarce. This contrast can be explained by the fact that studies of the latter type are relatively hard to achieve and take much material and money. Low (1982) attempted to calculate the amount of reabsorbed endogenous nitrogen during the passage of digesta through the pig's digestive tract. He based his calculations on various reports on endogenous secretion of the intestinal wall and its exocrine glands and on the nitrogen content of chyme in various parts of the gastro-intestinal tract. Low thus calculated that a daily intake of 40 g nitrogen results in an endogenous secretion of 18.1 g N, 78% of which ist reabsorbed before the terminal end of the large intestine. Although the latter value is somewhat theoretical it fairly agrees with results directly obtained in animal trials by other authors. Reabsorption of endogenously secreted nitrogen can be determinded only by combining several experimental methods and by using the tracer technique. Souffrant et al. (1986) reported on a combined trial with pigs which yielded data on endogenous pancreatic and biliary secretion as well as ileal and postileal absorption. In the same study they obtained data on reabsorption of endogenous nitrogen in the digestive tract too (Figure 1). In their studies casein was used as the sole protein source. Using the blood flow method and measuring the porto-arterial concentration differences they found a total N absorption of 29.7 g for a single N intake of 23.6 g. They could demonstrate by means of the ^{15}N dilution method that all of the casein fed had been re-absorbed in the terminal ileum and that the amount of nitrogen (2.2 g) found in the chyme of the terminal ileum was exclusively of endogenous origin. These data served to balance the recycling of endogenous nitrogen. Souffrant et al. found that a total amount of 7.4 g nitrogen had been secreted endogenously where of the re-absorption rate was 70% up to the terminal ileum and 82% up to the rectum.

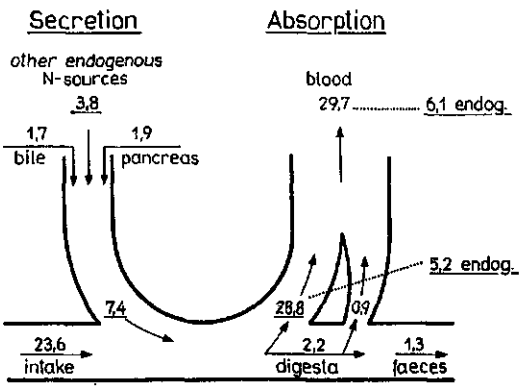

Fig. 1. Recycling scheme for endogenous nitrogen in pigs, in g N (After Souffrant et al. 1986).

Krawielitzki et al.(1990) have reported on another combination of methods which enables to measure the endogenous proportion of nitrogen in chyme at different parts of the gastro-intestinal tract and the endogenous nitrogen secretion in various intestinal sections. They used three pigs fitted each with re-entrant cannulae in the duodenum and the terminal ileum. The N pool of one animal was labelled with ^{15}N. By means of chyme exchanging between the pigs and based on the isotope dilution method, both the nitrogen flow and the secretion and reabsorption of endogenous nitrogen could be calculated for each section of the digestive tract. Soybean meal was used as the protein source. They found a total endogenous N secretion of 16.1 g for a daily N intake of 35 g. Up to the duodenum 33% of this nitrogen amount was secreted into the lumen of the digestive tract. Endogenous nitrogen secretion between the proximal and terminal small intestine accounted for 56% of total endogenous nitrogen secretion, whereas the proportion of endogenous nitrogen secreted in the large intestine was 11%. The reabsorption rate for the nitrogen secreted endogenously up to the terminal ileum was found to be 73%; for the whole of the digestive canal this rate was 90 %.

Although the results found are almost identical for both experimental set-ups it cannot be concluded that these values are invariables. In further trials the parameters of recycling of endogenous nitrogen should be investigated upon a diet of poorly digestible proteins.

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EXOCRINE PANCREATIC SECRETION OF ENZYMES IN GROWING PIGS GIVEN DIETS BASED ON BARLEY, PEA, LUPIN OR FIELD BEAN

Lucyna Buraczewska[1], Ute Pöhland[2], Jolanta Gdala[1], W. Grala[1], Grażyna Janowska[1] and W.B. Souffrant[2]

[1]Institute of Animal Physiology and Nutrition, Jabłonna, Poland
[2]Research Centre of Animal Production, Department of Animal Nutrition, Dummerstorf-Rostock, Republic of Germany

Abstract

Four pigs, initially 33-39 kg live weight, were prepared with a duodenal pouch for collection of pancreatic juice to assess the influence of feeding pigs with pea, lupin and field bean, as compared to barley, on exocrine pancreatic secretion. Four isonitrogenous (about 10.5% of N x 6.25) diets were fed to each pig: diet B (barley), diet BP (1:1 N of pea and barley), diet LB (1:1 N of lupin and barley) and diet FS (field bean and wheat starch). Mean volume of pancratic juice was 3.9 l, when expressed per 1 kg of OM intake, and was not affected by diet changes. The average daily protein, trypsin, chymotrypsin and amylase output was similar for B, PB and LB diets, but significantly lower for FS diet. Lipase secretion was lowered by feeding B and FS diets. Protein secretion (g/h) and trypsin and chymotrypsin activities of pancreatic juice (U/ml or h) were decreasing with time after a meal. Measurements of specific enzyme activities (U/mg protein) showed significantly higher trypsin activity in pigs fed PB than the other diets and comparatively the highest chymotrypsin specific activity for LS diet. It was concluded that the volume of pancreatic juice and its enzyme output were not appreciably altered by PB and LB diets, as compared to B diet. There is no clear explanation for decreasing effect on enzyme secretion of the FS diet, fed to pigs as a semi-synthetic one.

Keywords: pancreatic secretion, pigs, pea, field bean.

Introduction

Legume seeds contain substances which are referred as antinutritional factors, because they interfere with nutrient availability or cause other undesirable effects. Mostly known are trypsin inhibitors, lectins, tannins and alkaloids. Liener and Kakade (1989) summarized data regarding the effect of trypsin inhibitors on the pancreas in different animals showing that no pancreas hipertrophy occurs in larger animals like the pig and the calf. However, ingestion of raw, soybean diet by the pig induced an overall increase in volume of pancreatic juice, as compared to a heated soybean diet, and led to unsignificant increases in total protein and trypsin activity (Corring et al., 1985; Żebrowska et at., 1985). It is possible that a feedback mechanism (Corring, 1974) is involved in the response of the exocrine pancreas to feeds containing antinutritive components such as trypsin inhibitor or tannins. There are no evidences whether other than soya legumine seeds may influence pancreatic secretion of pigs. This experiment was designed to study the effect of feeding growing pigs with pea, field bean and lupin, as compared to barley, on the

volume and enzyme activity of pancreatic juice.

Materials and Methods:

Four female pigs of 33-39 kg live body mass were equipped with a re-entrant cannula linking a duodenal pouch with the duodenum. The surgical procedure was similar to that described by Hee et al. (1985).

Four diets were prepared (Table 1), each containing ten per cent of protein from different sources: barley, white-flowered pea "Opal", yellow lupin "Topaz" and field bean "Dino". Diets were supplemented with most limiting amino acids. From about 10th day after surgery the pigs were fed according to their metabolic body weight.

Table 1. Composition of diets*.

Ingredients, g/kg	Diets with: barley	pea	lupin	field bean
Barley	959.5	-	-	-
Pea, var. "Opal"	-	523.0	-	-
Yellow lupin, var. "Topaz"	-	-	265.0	-
Field bean, var. "Dino"	-	-	-	425.0
Wheat starch	-	415.8	686.3	515.3
Cellulose	-	6.0	-	4.0
Dicalcium phosphate	10.0	16.0	18.0	15.0
Calcium carbonate	15.0	10.0	8.0	11.0
Vit.-min. mixture, "Polfamix P"	10.0	10.0	10.0	10.0
Sodium chloride	3.0	4.0	4.0	4.0
Soya oil	-	13.9	8.6	14.7
Lysine HCl**	2.45	-	-	-
Methionine	-	1.26	-	0.84
Tryptophan	-	-	0.11	-

* Pea and lupin diets were mixed with barley diet 1:1 to increase the intake of the feed by pigs. Protein and tannin contents in the diets were as follows: pea-barley diet 10.7 and 0.19%, lupin-barley diet 10.8 and 0.17% and field bean 10.6 and 0.56%, respectively. NDF content in the ingredients was: in barley 14.5%, in pea 12.3%, in lupin 20.6% and in field bean 13.4%.

**No Lys was added to the pea-barley mixture

It is worth to note that the meals of legume seeds were very well accepted by pigs when fed with barley during preliminary checking of their palatability. Replacing barley by starch in experimental diets reduced the intake of pea and lupin diets, and it appeard necessary to feed these diets with the barley diet in proportion 1:1. At the beginning of the experiment the pigs were fed the experimental diets according to Latin square design. The diets were given to the pigs twice daily, at 8.00 and at 20.00 h in equal portions after mixing with water (about 1:1) immediately before feeding. Water was available at all times. Each pig received each diet for 14 days.

Pancreatic juice was collected every 2 week on days 12 and 14 of feeding the same diet, each time for 24 h (from 8.00 to 8.00 h). The weight

of juice was measured every hour and 5-10% samples were taken for pool samples representative of the three consecutive periods: 2, 6 and 4 hours after each meal. At the same time daily samples for each pig, each diet and each day of collection were bulked. The samples were stored at 0°C to await analysis. The remaining juice was reintroduced by means of peristaltic pump at a rate similar to the outflow. Four enzymes and protein were determined in the samples next day after the juice collection.

Protein was estimated by the method of Lowry et al. (1951) using porcine serum albumine as a reference. Trypsin and chymotrypsin were measured according to Hummel (1959) and amylase according to Bernfield (1951). The procedure used for lipase analysis is based on the method of Tietz and Fiereck (1966). Activity of lipase was expressed in Sigma-Tietz Units which are equal to the ml of 0.05 N NaOH required to neutralize the fatty acids liberated during 6 hours of incubation with 3 ml lipase substrate, according to Sigma Diagnostics. For estimation, 1 ml of the juice was diluted 50 folds with Tris buffer 0.2 M, pH 8.0. Lipase and also amylase were estimated only in the daily samples.

Results and Discussion

The juice was dripping continuously and dietary treatment has no significant effect on its volume secreted in 24 h (Table 2) which was in average about 3.9 l when expressed per 1 kg dry matter intake of all the diets. However, large variations in volume of pancreatic juice were observed for each diet, both between pigs and between the two days of collection. As it was presented by Hee et al. (1985) considerable variation (1.2 to 5.0 l) has been reported in the literature, for daily volume of pancreatic juice collected from growing pigs. Values observed in the present studies, for four pigs during their growth from 33 to 74 kg, fall within the upper range of the reported volumes and did not prove any tendency for an increase of juice secretion by the leguminous feeds.

Table 2. Mean daily output of pancreatic juice and some of its enzymes expressed per 1 kg DM intake of different diets.

	Diets*			
	barley	pea + barley	lupin + barley	field bean
Pancreatic juice, ml	3752	4142	3721	3918
Trypsin, U x 10^{-3}	163.8^{a}	170.6^{A}	172.6^{A}	117.3^{Bb}
Chymotrypsin, U x 10^{-3}	49.8^{a}	46.8^{a}	63.4^{A}	28.3^{B}
Amylase, U x 10^{-3}	963^{a}	631^{a}	748^{a}	466^{b}
Lipase, U x 10^{-3}	746^{ac}	1159^{b}	1139^{bc}	693^{a}

* For details of diets, see Table 1. Means with different superscript letters in between diet comparisons differ significantly (a,b,c $P<0.05$; A, B $P<0.01$)

The mean volume of pancreatic juice secreted during the first and the second 12 h periods after the meals given at 8.00 and 20.00 h were similar, and this was so far each of the diet. The hourly volumes of juice

were very variable, and showed no clear response to feeding and no consistant diurnal pattern for the diets, what was consistant with some other findings (Partridge et al., 1982; Żebrowska and Długołęcka, 1988).

Mean values for enzyme activity (U/24 h) of pancreatic juice presented in Table 2 show that tryptic, chymotryptic and amylolytic activities were not appreciably altered after the pea-barley and lupin -barley diets, as compared to the barley diet. The only significant difference was found for lipase activity which was lower for barley than for the barley--legume seed diets. Moreover, all the barley containing diets affected significantly higher daily output of the enzymes than the semi-synthetic field bean diet. Similar dietary relations were also found for protein secretion (Table 3).

Table 3. Rate of protein secretion in pancreatic juice collected during three cosecutive periods (2, 6 and 4 h) after meals given every twelve hours, at 8.00 and 20.00 h. Values are expressed per 1 kg DM intake of each diet.

Collection period	Protein/ time	Diets			
		barley	pea + barley	lupin + barley	field bean
Periods after the meals:					
2 hours	g/2 h	1.26	1.06	1.53	1.06
6 hours	g/2 h	1.00^{a}	0.91^{a}	0.98^{a}	0.67^{b}
4 hours	g/2 h	0.84	0.72	0.76	0.61
Daily *	g/24 h	12.94^{a}	10.59^{a}	12.75^{Ab}	8.76^{Bb}

*Protein as N x 6.25. Means with different superscript letters in between diet comparisons differ significantly (A,B $P<0.01$ or 0.001; a,b $P<0.05$)

These changes, both in protein and in enzyme daily secretion, might have been elicited by changes in the type of dietary fiber or/and, to some extent, by differences in proteins and starches of the diets. However, this does not exclude a possibility of depressing effect of antinutritional factors of the field bean seeds. Recently, Hasdai et al. (1989) found that the pancreas of guinea-pigs fed raw or heated soya-bean flour was similar in weght but secreted less trypsin, chymotrypsin and amylase in raw soya-bean fed animals. The authors concluded that the mechanism, by which raw soya-bean negatively effects the activity of enzymes, differs from that suggested for rats and chicks but is similar to that of pigs and calves.

No significant differences were observed in trypsin and chymotrypsin activity in pancreatic juice secreted during the day or the night 12 h periods and the pattern of enzyme secretion was similar after the morning and after the evening meals.

Protein secretion (Table 3) and trypsin and chymotrypsin activities of pancreatic juice (Table 4) were decreasing with time after a meal. It is proved that protein or enzyme-reach juice is secreted in response to products of protein digestion passing the duodenum, whereas digesta

of low pH stimulates secretion of low protein pancreatic juice (Harper, 1967). Studies in pigs have shown that up to 70% of dietary protein may leave the stomach during the first 5-6 h after a meal with a rate continuously decreasing, and much of this can be in the form of peptides (Żebrowska and Buraczewska, 1972).

Table 4. Trypsin and chymotrypsin activity expressed in U per 1 ml of pancreatic juice collected during three consecutive periods (2, 6 and 4 h) after the meals given every twelve hours.

Consecutive periods, h	Diets: barley	lupin + barley	pea + barley	field bean
	Trypsin activity			
2	53.1±13.1	68.6±18.5	50.1±20.0	56.7±13.9
6	41.7±10.0	44.7±11.4	40.9±12.0	28.5± 4.1
4	36.8±12.6	37.6±10.0	34.2± 7.0	30.0± 7.0
	Chymotrypsin activity			
2	16.6± 4.5	26.9± 4.3	13.2± 6.0	15.2± 3.1
6	13.1± 3.5	16.1± 3.9	11.8± 6.4	7.6± 3.0
4	11.4± 3.4	12.7± 4.0	8.8± 3.2	5.4± 1.6

The ratio of enzyme activities to protein in pancreatic juice was constant in all the collected samples after a diet. Calculated means of specific activity of enzymes (U/mg protein), on the basis of results for three consecutive periods (2, 4 and 6 h) after the meals, showed (Table 5) significantly higher trypsin specific activity in pigs fed pea-barley diet than the other diets, and also significant differences in chymotrypsin specific activities; the highest for lupin-barley diet and the lowest for field bean diet.

Table 5. Means of specific activity of trypsin and chymotrypsin (U/mg protein) in pancreatic juice collected during three consecutive periods (2, 6 and 4 h) after the meal given every twelve hours.

Enzymes	Diets: barley	pea + barley	lupin + barley	field bean
Trypsin	13.53^{A}	16.21^{B}	13.90^{A}	13.49^{A}
Chymotrypsin	4.27^{A}	4.33^{A}	4.93^{B}	3.18^{C}

Means with different superscript letters in between diet comparisons differ significantly; $P<0.01$ or 0.001

Conclusions

The results of the present study indicate that, under conditions of constant total dietary protein intake for about 5 per cent of barley and about 5 per cent of pea or lupin protein, pancreatic enzyme output was

not significantly affected , as compared to 10 per cent barley protein in a diet. Consumption of the semi-synthetic diet containing 10 per cent of field bean protein significantly lowered pancreatic secretion of all the measured enzymes.

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EVALUATION OF A METHOD TO STUDY PROTEIN DIGESTION IN THE PROXIMAL DIGESTIVE TRACT OF YOUNG PIGLETS

C.A. Makkink[1], B. Kemp[1], J.M. Fentener van Vlissingen[2]

1 Agricultural University, Department of Animal Nutrition, Haagsteeg 4, 6700 PM Wageningen, The Netherlands

2 TNO-Institute of Animal Nutrition and Physiology, P.O. Box 15, 6700 AA Wageningen, The Netherlands

Introduction

The digestion of different protein sources in young piglets is easily disturbed: certain (plant) proteins (e.g., soy protein) are less digestible than other (animal) proteins (e.g., milk protein). This difference in protein digestibility is much less pronounced in older pigs (Wilson & Leibholz, 1981a,b,c).
Digestive disorders in young piglets could be due to lack of digestive enzymes or to problems with pH regulation in the proximal digestive tract (stomach and pancreas).
In trial 1, cannulation of the duodenum was performed to measure pH, protein breakdown and proteolytic enzyme activities in duodenal digesta. The cannulation technique was evaluated with regard to sustainability of the animal model, growth and feed conversion efficiency of the piglets.
In trial 2, piglets of the same age were used and they were fed the same diets as in trial 1 were. They were not cannulated and served as control group. Afterwards, these piglets were fitted with a pancreatic duct cannula so that pancreatic juice could be measured.

Experimental Design

Trial 1

Two diets were prepared (Table 1) containing either skimmilk powder (SMP) or soybean meal (SBM) as the sole source of protein. Six piglets were weaned at 4½ weeks of age and fed either the SBM or the SMP diet. Piglets were fitted with a simple duodenal T-cannula at 5½ weeks of age. Three digesta collection periods of 4 days each were planned according to the following scheme:

	period	I	II	III
	age (weeks)	6½	8½	10½
pigletnr	1 and 2	SMP	SMP	SBM
	3 and 4	SMP	SBM	SBM
	5 and 6	SBM	SBM	SBM

Piglets were fed at 8:00am and 16:30pm. Daily ration was 400 grams, 500 grams and 600 grams in period I, II and III, respectively. In each collection period duodenal digesta samples were taken at hourly intervals between 8:30am (½ hour after feeding time) and 16:00pm, during four days per period.

The pH of the samples was measured immediately after collection. One gram of each sample was mixed with trichloroacetic acid (TCA) for the analysis of TCA-precipitable protein. The remainder of the sample was frozen and freezedried for crude protein and enzyme analyses. Samples taken at 12:00 (4 hours after feeding, when pancreatic juice was maximal, Makkink, unpublished results) were analyzed for protease activity. After the third collection period, piglets 2 and 5 were anaesthetized (O_2, N_2O, halothane) after the morning feeding and fitted with a catheter in the pancreatic duct. Pancreatic juice was collected from 1.5 upto 6.5 hours after feeding (basal secretion). Then a secretin injection (0.1 Clinical Units/kg LW, intravenously) was given to measure maximal pancreatic juice output. After secretin injection pancreatic juice was collected during the following 30 minutes. Pancreatic tissue was removed and weighed after killing the animal.

Trial 2

Five piglets were fed one of the two diets (Table 1) after weaning at 4½ weeks of age. Piglets 7, 8 and 9 were fed the SBM-diet, piglets 10 and 11 were fed the SMP-diet.
At 8 weeks of age, piglets were anaesthetized and fitted with a pancreatic duct catheter. Pancreatic juice was collected as described for trial 1.

Table 1. Composition of the diets

ingredient	diet SMP	diet SBM
soybean meal (47.5 % CP)	-	34.40
skimmilk powder (35.3 % CP)	45.50	-
maize starch	29.60	39.84
dextrose	15.00	15.00
sunflower/soy oil	2.00	2.00
cellulose	5.00	2.85
vit/min premix	1.00	1.00
ground limestone	0.80	1.35
mono Ca phosphate	0.50	2.10
salt	-	0.50
$KHCO_3$	0.10	-
$NaHCO_3$	0.30	0.40
L-Lysine HCl	-	0.16
DL-Methionine	0.10	0.20
L-Threonine	-	0.10
Cr_2O_3	0.1	0.1
calculated contents		
crude protein	16.08	16.37
digestible CP	15.44	14.76
net energy (kcal/kg)	2555	2477
crude fat	2.49	2.44
crude fiber	4.95	5.02
inorganic matter	5.33	5.88

Results

In trial 1, three piglets were killed during the experiment because of complications after surgery. Piglet 6 (SBM) was killed before the start of period I (peritonitis). Piglets 3 and 4 were killed during period II, after being put on the SBM diet (local peritonitis + spastic ileus and entrapment of the jejunum through a hernia in the meso-duodenum, respectively). Thus, only 50 % of the cannulated piglets survived throughout the experiment. In trial 2, no health problems were experienced. Growth, feed intake and feed conversion ratios from both trials are presented in Table 2.

Table 2. Growth, feed intake and feed conversion ratio in trials 1 and 2.

		growth (g/d)	feed intake (g/d)	feed conversion ratio
trial 1				
period I	piglet 1+2	250 ± 81	400	1.69 ± 0.54
	piglet 3+4	266 ± 48	400	1.53 ± 0.28
	piglet 5	186	400	2.15
period II	piglet 1+2	232 ± 86	500	2.31 ± 0.86
	piglet 3+4	-	-	-
	piglet 5	193	500	2.59
period III	piglet 1+2	241 ± 280	600	7.68 ± 8.93
	piglet 5	271	600	2.21
trial 2				
period I (week 1)	piglet 7,8+9	201 ± 43	400	2.26 ± 0.41
	piglet 10+11	196 ± 56	400	2.12 ± 0.60
week 2	piglet 7,8+9	163 ± 24	400	2.49 ± 0.40
	piglet 10+11	211 ± 5	400	1.90 ± 0.05

Pancreas weight was related to body weight in both trials according to the following regression equation:

$Y = 2.84 * X - 13.77$

Y = weight of pancreas (grams)
X = body weight (kg)
r = 0.96
n = 8

Protein source in the diet did not influence this relation. Results from piglets in trial 1 showed that pH in duodenal digesta decreased ± 1 pH unit between 0 and 4 hours after feeding and increased thereafter. pH was not affected by protein source. Protein breakdown tended to increase with advancing age of the piglets (Figure 1). An effect of protein source could not be demonstrated.
Proteolytic enzyme activities in duodenal digesta from piglets in trial 1 varied enormously between days, between periods and between piglets. Trypsin activity varied between 5 and 1829 units per gram dry matter, chymotrypsin activity varied between 0.2 and 511 units per gram dry matter. The trypsin/chymotrypsin

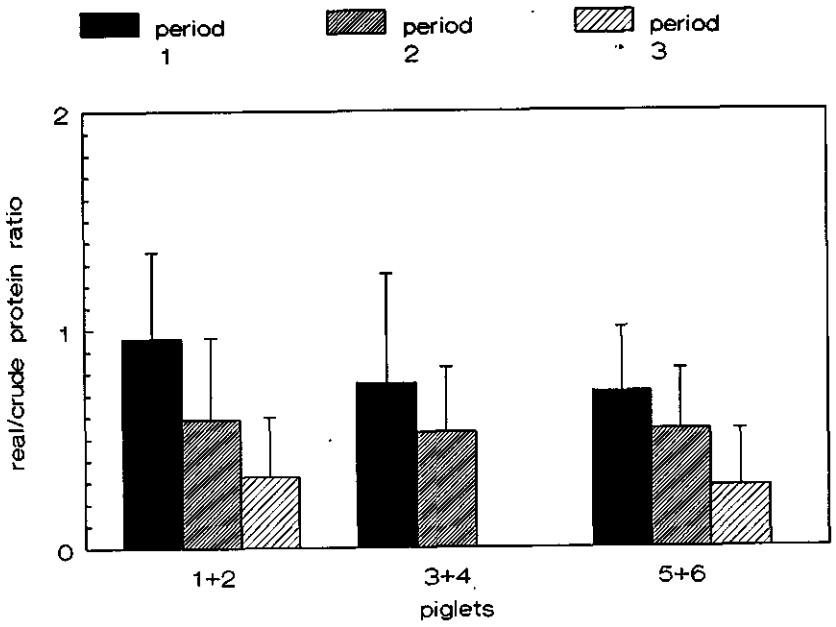

Figure 1. Real/crude protein ratio in duodenal digesta.

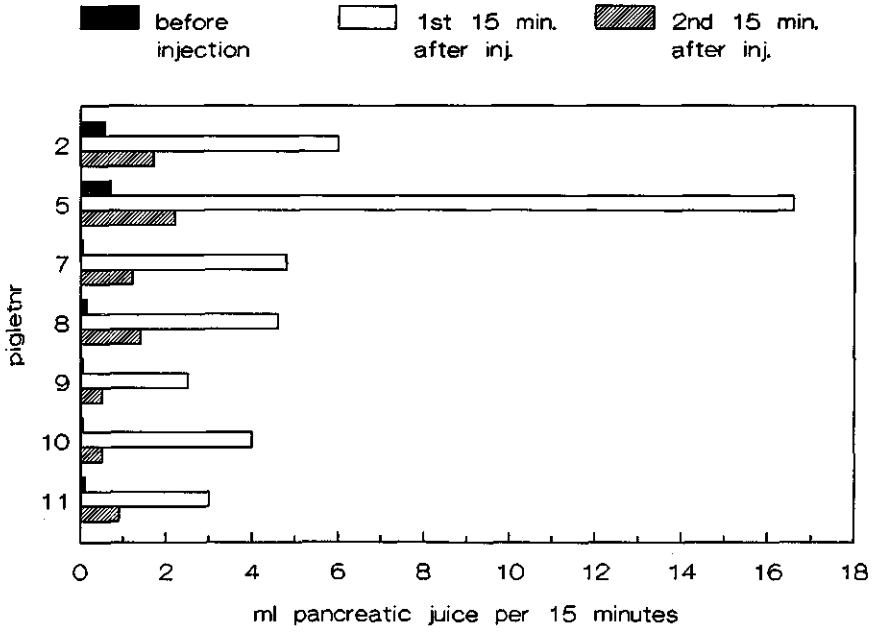

Figure 2. Pancreatic juice secretion before and after secretin injection.

ratio tended to be somewhat higher for piglets fed the SBM diet, however, no significant differences between treatments were found.
The basal and secretin-stimulated pancreatic juice secretion (trial 1 + 2) is presented in Figure 2. Basal secretion ranged from 0.2 to 2.8 ml per hour (n=7). All piglets clearly responded to the secretin administration, juice outflow during the first 15 minutes after injection ranging from 10.0 to 66.4 ml/h. During the second 15 minutes after injection, pancreatic juice flow ranged from 2.0 to 8.8 ml/h. With all piglets, pancreatic outflow was stimulated for at least 30 minutes after secretin injection. No significant differences between diets could be demonstrated.

Discussion

Cannulation of young piglets at the site of the duodenum is possible although health risks are encountered.
The course of the experiment after cannulation of the duodenum was complicated in these young piglets. In this region, just after the pylorus, digesta flow rate is relatively high. Therefore, digesta sampling must be performed using the spot sampling technique (cannula open during short time periods). When the cannula is opened for more then a few minutes, stomach emptying will be stimulated, causing unphysiological changes in transit time. During the collection of pancreatic juice under anaesthesia, gastric emptying was disturbed.
In this experiment no significant differences were found between protein sources with regard to pH, protein breakdown or proteolytic enzyme activities in duodenal chyme.
Protein breakdown tended to increase with advancing age of the piglets (trial 1), indicating a developmental increase in proteolytic capability of the proximal digestive tract of piglets. Interactions between age and protein source (differences in adaptation between the two protein sources) could not be demonstrated.
Fistulation of the pancreatic duct can be performed in young piglets (± 8 weeks of age) and measurement of pancreatic secretion before and after hormonal stimulation is possible. Differences in basal pancreatic secretion rate were detected between piglets in trial 1 and trial 2. However, these differences were not related to protein source in the feed. The response to secretin injection was very clearly demonstrated in all piglets (Figure 2). Not enough data were collected to demonstrate differences between treatments (age and protein source). However, differences in stimulated secretion rate were found between piglets, indicating a difference in developmental stage with regard to pancreatic secretory capacity. More experiments of this type will be performed to investigate the effects of dietary protein sources and age of piglets on the development of the pancreas.

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THE PROBLEM OF ESTIMATION OF AMINO ACID DIGESTIBILITY IN PIGS

H. Bergner

Institute of Nutrition Physiology of the Humboldt University Berlin

Abstract

The so-called "amino acid digestibility" is a balance of the amino acids in the whole digestive tract or of a part of the digestive tract. The bacterial degradation and synthesis of amino acids falsify in the large intestine and also in the small intestine these balances. The high microbial synthesis of lysine and the small synthesis of histidine make it possible to value this influence on the amino acid digestibility. The best method for the measurement of the non feed amino acids at the end of the ileum or in the faeces is the 15N-isotope dilution technique with 15N-labelled pigs.

Apparent digestibility of amino acids in the whole digestive tract of pigs.

In 1982 we published (Bergner, 1982) that the faecal apparent digestibility of the most amino acids is in dependence from the crude fibre content of the diet. From this experiments the apparent digestibility for lysine, histidine and proline is shown in Table 1.

Table 1. Faecal apparent digestibility (%) of the amino acids lysine, histidine and proline in dependence of the crude fibre content (Bergner, 1982).

% of crude fibre in the diet	4.2	6.3	8.0	11.1	13.5
Lysine	83	81	75	69	63
Histidine	90	89	85	82	77
Proline	96	94	92	90	88

Further experiments showed (Bergner et al., 1984) that the excretion of 15N-labelled amino acids in the faeces of 15N-labelled pigs was the highest for 15N-lysine and the lowest for 15N-histidine and 15N-proline. The results showed further that the increase of excretion of 15N-lysine was positively correlated with the increase of crude fibre in the diet. This correlation was very small for the excretion of 15N-labelled histidine and the smallest for

15N-labelled proline. The reason for this result is the high bacterial synthesis of lysine and the low bacterial synthesis of histidine and proline. The results in Table 1 are in agreement with this explanations. When the bacterial synthesis rate of an amino acid is very high, the apparent digestibility (balance) of this amino acid is low, and inverted, when the bacterial synthesis of an amino acid in the gut is low the apparent digestibility is high.

Ileal apparent and true digestibility of amino acids in pigs.

We postulate that the bacterial synthesis of amino acids in the small intestine of pigs is also responsible for the apparent ileal digestibility when enough native fibre is included in the diet as an energy source for the bacterial activity in the distal part of the small intestine.

The results of Zebrowska et al. (1984) showed for the apparent ileal digestibility of lysine and histidine the picture what is given in Table 2.

Table 2. Apparent ileal digestibility in pigs (%) of lysine and histidine of diets with natural fibre (Zebrowska et al., 1984).

Diet	Barley	Wheat	Rye	Oats	Wheat bran
Lysine	66.4	62.3	65.3	58.1	57.1
Histidine	80.5	79.9	75.7	74.4	67.2

The higher lysine synthesis rate caused the higher values of lysine at the end of the ileum and the lower apparent digestibilitys for lysine in comparison to histidine.

The calculation of a true ileal digestibility of amino acids considering the endogenous amino acids at the end of the ileum is problematical in a protein-free feeding experiment. The protein-free diets contain cellulose as a fibre source. The cellulose is contrary to the pentosans of the grains not so a good energy source for the gut bacteria. A second problem during the feeding of a protein-free diet is the lower influx of non protein nitrogen (urea) in the small intestine and the bacterial amino acid synthesis is reduced.

The values of apparent and true ileal amino acid digestibilities are shown in Table 3 for diets with cellulose. The high apparent digestibility of lysine indicated in this experiment a low bacterial activity in dependence from the cellulose diet. The larger amount of lysine at the end of the ileum in presence of natural fibre in the diet is not an effect of a higher transport rate through the small in-

testine for lysine against histidine (Table 1 and 2), because Herrmann et al. (1988) showed that the supplement of wheat straw meal or grass meal increased not the transport rate of histidine or phenylalanine (lower digestibility) to the end of the ileum as shown in Table 4.

Table 3. Apparent and true ileal digestibilies in pigs (%) of lysine and histidine of diets with cellulose as a fibre source (Poppe et al., 1983).

	Apparent digestibility		True digestibility	
Diet	lysine	histidine	lysine	histidine
6 % casein	87.6	86.8	99.9	98.9
11 % casein	93.8	95.5	99.9	100.0
18 % casein	93.6	95.0	97.7	99.2
24 % casein	93.1	93.2	95.9	96.0

Table 4. Apparent ileal digestibility in pigs (%) of lysine, histidine and phenylalanine of diets with different natural fibre (Herrmann et al., 1988).

	Lys	His	Phe
Basis diet (BD) (88 % wheat, 6 % soyabean meal, 4 % fish meal)	71.1	83.6	83.2
BD incl. 9.8 % wheat straw meal	70.2	85.1	79.8
BD incl. 16.7 % wheat straw meal	69.1	83.5	81.7
BD incl. 10.0 % grass meal	70.7	83.8	83.0
BD incl. 18.1 % grass meal	64.5	82.1	79.3

We found in our experiments to the adsorptive effects of amino acids to food constituents a high connection between the aromatic rings of phenylalanine and the ring polymers of isolated lignin or straw meal (Bergner et al., 1981). Straw meal adsorbed 10 % phenalalanine (weight/weight). The bacterial phenylalanine synthesis in the hindgut was found by means of the 15N-incorporation in phenylalanine, something higher as the bacterial histidine synthesis (Bergner et al., 1984). The low adsorption effect between straw meal and phenylalanine in the small intestine is caused through the high water content of the digesta in this part of the digestive tract. The high bacterial lysine synthesis (Table 4) is in agreement with the high ileal apparent digestibility of hemicelluloses in the small intestine in the same

experiment (Herrmann et al., 1988).

When a bacterial activity exist in the small intestine, amino acids are synthetisized and destroid. The measurement of non feed amino acids at the end of the ileum is possible in 15N-labelled pigs with the 15N-isotope dilution technique.

De Lange et al. (1988) estimated the endogenous protein (non feed protein) in the ileal digesta with the 15N-isotope dilution technique in pigs (38 kg LW). The results are shown in Table 5.

Table 5. Endogenous protein in ileal digesta of pigs (de Lange et al., 1988).

Diet	Wheat	Barley
Endogenous protein		
per kg DM intake	27.4 g	27.7 g
per 100 g crude protein intake	19.1 g	24.7 g
% of total crude protein	94.5	81.1

This endogenous protein is real endogenous protein (enzymes, epithel cells etc.) and bacterial protein. De Lange et al. (1988) (see also Souffrant et al., 1990) found in this experiment true ileal digestibilities for histidine and lysine of wheat and barley diets in the range of 97 to 100 %. In such experiments the amino acids of feedstuffs are at the end of the ileum non 15N-labelled and the endogenous and bacterial amino acids are 15N-labelled. It is to conclude that all methods for the estimation of apparent or true ileal digestibility of amino acids are insufficient. The best method of the measurement of amino acid digestibility is the 15N-isotope dilution technique with 15N-labelled pigs.

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GASTRIN SECRETION IN THE NEONATAL PIG IN RESPONSE TO THE INTRODUCTION OF EITHER COLOSTRUM OR A PEPTONE SOLUTION INTO THE STOMACH

R-J. Xu[1] and P.D. Cranwell

School of Agriculture, La Trobe University, Bundoora, Victoria 3083, Australia

Abstract

Hepatic-portal and peripheral (jugular v) plasma gastrin concentrations were measured in pigs aged 4-6 hours (unsuckled, n = 10), 6 days (fasted for 18 hours, n = 8) and 12 days (fasted for 18 hours, n = 7) before and after the introduction of colostrum or a 10% peptone solution or physiological saline into the stomach (8ml/kg body-weight) for a period of 60 minutes. In all pigs hepatic-portal plasma gastrin concentrations prior to the introduction of either colostrum or peptone solution into the stomach were significantly greater than in peripheral plasma. Following the introduction of the peptone solution there was a significant increase in both the hepatic-portal and peripheral plasma gastrin concentrations in pigs of all ages. After the introduction of colostrum significant increases in hepatic-portal and peripheral plasma gastrin concentrations occurred only in 12-day-old pigs. The results indicate that gastrin secretion is stimulated during the birth process, that G-cell secretion of gastrin in response to stimulation by a mixture of amino acids and peptides occurs in the newborn unsuckled pig, and that colostrum does not evoke a gastrin response in pigs younger than 1 week of age.

Keywords: gastrin; neonatal pig; colostrum; peptone solution; stomach; G-cells

Introduction

The increase in gastrin secretion in response to a meal has been well documented in adult animals (Walsh & Grossman, 1975). When food is eaten gastrin is released as the result of cephalic vagal stimulation, gastric distension and dietary chemical stimulation of the antral G-cells. The release of gastrin is inhibited by a negative feedback mechanism via gastric acid secretion and low gastric antral pH. Information regarding the regulation of gastrin secretion in neonatal animals however, is sparse and somewhat inconclusive. For instance, evidence for a gastrin response to feeding in human neonates is conflicting. Sann et al. (1975) and von Berger et al. (1976) reported that plasma gastrin increased significantly after human newborns and infants were fed with a milk formula; whereas Rodgers et al. (1978) and Moazam et al. (1984) did not observe such increases in human newborns and infants after feeding with a milk formula or breast milk. Similarly, studies with pigs have also produced

[1] Present address: Department of Physiology and Anatomy, Massey University, Palmerston North, New Zealand.

somewhat confusing results. For example, Moazam et al. (1980) did not observe a significant increase in serum gastrin following the introduction of a protein solution into the stomach of pigs 1-4 weeks old, and Bunn & Titchen (1984) reported a significant decrease of serum gastrin in newborn pigs after the first sucking. The aim of the present study was to determine if neonatal pigs secrete gastrin in response to the introduction into the stomach of either colostrum or a peptone solution.

Materials and Methods

Twenty five Large White x Landrace pigs from 4 litters, <1-12 days old, 0.8-3.8 kg body-weight were used in this study. Immediately after birth arterial blood samples were obtained from 19 pigs (3 litters) by transecting the umbilical cord 3-5 cm from the navel, collecting a 5 ml blood sample and then clamping the cord with a sterile cord clamp. Ten newborn pigs, which had not sucked from the sows, were used within 4-6 hours of birth. The remaining 15 pigs, reared entirely by the sows, were fasted for 15-20 hours prior to experiments at 6 (n=8) and 12 (n=7) days of age.

Anaesthesia was induced and maintained throughout the experiments with halothane, which was first inhaled through a mask, using an initial concentration of up to 5% in oxygen. A midline incision was made in the neck and an endotracheal tube was tied into the trachea through which the animal inhaled the anaesthetic at a concentration of 1-3% in oxygen. A silastic tube (2 mm i.d.) was tied into the oesophagus so that the tip was adjacent to the gastro-oesophageal junction. A further incision was made in the neck and a polyvinyl catheter (1.0 mm i.d.) was tied into the right external jugular vein. The abdomen was opened through a midline incision and a polyvinyl catheter (0.8 mm i.d.) was tied into the umbilical vein such that the tip of the catheter was in the hepatic-portal vein. During the first two weeks of life in the pig, it is possible to use this procedure to obtain samples of hepatic-portal blood without extensive surgical interference to the hepatic-portal vasculature. However, it should be stressed that the blood samples are a mixture of blood derived from all parts of the gastrointestinal tract, not just the stomach.

A bolus of 8 ml/kg body-weight of sows' colostrum (SC), or peptone solution (PS), or physiological saline was introduced by syringe into the stomach via the oesophageal tube. The peptone solution consisted of 10 g Bacteriological Peptone L37 (Oxoid, Basingstoke, U.K.) dissolved in 100 ml distilled water. The colostrum was collected from sows immediately prior to, during and following farrowing and was stored at -20°C until used. Blood samples (1 ml) were collected simultaneously from the peripheral and hepatic-portal catheters at known intervals before and after the bolus. Plasma gastrin concentration was determined by radioimmunoassay using the antiserum RPN 1651 (Amersham, U.K.). The antiserum, which was raised in rabbits against synthetic G17, recognizes the mid-to-C-terminal region of G17 and has equal cross-reactivity with both the G17 and G34 forms of human and porcine gastrin. The intra- and inter-assay coefficients of variation were 6.1-8.6% and 11.9-14.1% respectively. Statistical analyses were performed using paired and unpaired Students t-tests and the least significant difference (LSD) test (Steel & Torrie, 1980).

Results

The mean plasma gastrin concentration in umbilical arterial blood collected at birth from 19 pigs was 45±9 fmol/ml. Eight of these pigs were used in experiments at 4-6 hours after birth and the gastrin concentration in their umbilical arterial blood at birth, 49±9 fmol/ml, was significantly greater ($P<0.05$) than in peripheral blood 4-6 hours later, 22±5 fmol/ml. In pigs of all ages, hepatic-portal plasma gastrin concentrations during the fasting period were significantly greater than in peripheral plasma (*Table 1*).

Table 1. Hepatic-portal and peripheral plasma gastrin concentrations (mean±SEM) in newborn, unsuckled pigs, 4-6 hours old, and in 6- and 12-day-old pigs following a 15-20 hour period of fasting.

Age	n	Hepatic-portal (fmol/ml)		Peripheral (fmol/ml)
4-6 h	10	37 ± 8^a	**	20 ± 4^a
6 d	8	33 ± 5^a	*	27 ± 5^a
12 d	7	47 ± 5^a	*	41 ± 4^b

Differences between the hepatic-portal and peripheral plasma gastrin concentrations were significant * $P<0.05$; ** $P<0.01$. Mean values in a column with different superscripts (a,b) were significantly different ($P<0.01$).

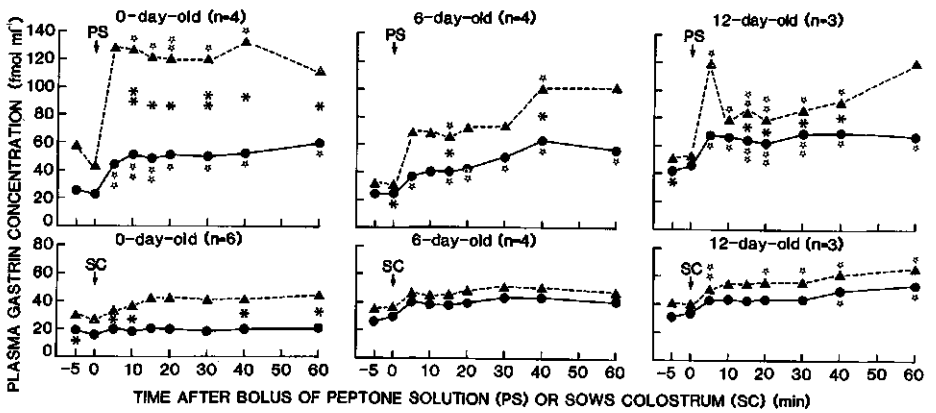

Figure 1. Hepatic-portal (▲) and peripheral (●) plasma gastrin concentrations in 4-6 hour old (0-day-old), and 6- and 12-day-old pigs before and after the introduction of a bolus of either peptone solution (PS) or colostrum (SC) into the stomach. Differences between the hepatic-portal and peripheral plasma gastrin concentrations were significant, * $P<0.05$; ** $P<0.01$. Differences between the hepatic-portal and peripheral plasma gastrin concentrations before and after the introduction of peptone solution or colostrum were significant, ☆ $P< 0.05$; ☆☆ $P < 0.01$.

Within 5-15 minutes of the introduction of PS into the stomach of pigs of all ages hepatic-portal and peripheral plasma gastrin concentrations increased significantly above fasting levels and remained elevated throughout the 60 minute period (*Figure 1*). In the pigs given PS, gastrin concentrations were always greater in hepatic-portal plasma than in peripheral plasma and the differences were often significant. Following the introduction of SC into the stomach, the hepatic-portal and peripheral plasma gastrin concentrations increased significantly above fasting levels only in the 12-day-old pigs. In all pigs which received SC, the differences between hepatic-portal and peripheral gastrin concentrations were small and only occasionally reached statistical significance. In one 12-day-old pig, gastrin concentrations in both hepatic-portal and peripheral plasma did not increase above fasting levels following the introduction of physiological saline into the stomach.

Discussion

Elevated blood gastrin concentrations in newborn pigs and human newborns, which often exceed the corresponding maternal blood concentrations, have been reported by von Berger et al. (1976), Rodgers et al. (1978), Cranwell & Hansky (1980a,b) and Marchini et al. (1988). In the present study, the gastrin concentrations in the peripheral blood of newborn pigs at birth were greater than those observed in the same pigs 4-6 hours later. A similar observation was made by Bunn & Titchen (1984) but their pigs were suckled in the intervening period. In human newborns, plasma gastrin concentrations decreased substantially during the first 2-6 hours of life and before the first feeding (Lucas et al. 1982). The higher concentrations of catecholamines in umbilical blood of human newborns delivered vaginally indicates that they undergo a greater degree of stress during the birth process than those delivered by caesarian section (Hägnevik et al. 1984). As gastrin concentrations in cord blood from vaginally delivered human infants are also higher than those delivered by caesarian section and since catecholamines are known to stimulate gastrin release (Marchini et al. 1988), it is therefore possible that the elevated circulating gastrin levels at birth result from stimuli arising from the stress of the birth process (Euler et al. 1977). These same factors may also be responsible for the elevated plasma gastrin concentrations in newborn pigs reported in this paper.

Introduction of peptone solution into the stomach evoked dramatic and significant increases in hepatic-portal and peripheral plasma gastrin concentrations in anaesthetized pigs at all three ages. Such responses are unlikely to be due to physical stimulation (gastric distension) alone since the introduction of an equivalent volume of colostrum (on a body-weight basis) into the stomachs of 0- and 6-day-old pigs and the introduction of an equivalent volume of saline into the stomach of a 12-day-old pig did not evoke significant increases in peripheral or hepatic-portal plasma gastrin concentrations. The response to peptone solution is more likely to be due to its chemical composition. It has been reported by a number of researchers that small peptides and amino acids, and their metabolic breakdown products, particularly amines and ammonia, are the most potent stimulants of gastrin release (Elwin, 1974; Lichtenberger et al. 1982; Lichtenberger, 1982). The

peptone (L37) used in the present experiments contains significant quantities of free amino acids and ammonia.

In contrast to the effects of the peptone solution, colostrum stimulated small increases in the hepatic-portal and peripheral plasma gastrin concentrations, and the increases were significant only in the 12-day-old pigs. The dramatic differences between peptone solution and the colostrum in their stimulatory effects on gastrin release are probably due to the differences in their chemical composition. Colostrum contains significant quantities of fat and carbohydrate (Cranwell & Moughan, 1989) but neither are effective stimulants of gastrin release (Korman et al. 1971; Richardson et al. 1976). Colostrum also contains about 15-16% protein which would not undergo significant gastric hydrolysis to form the metabolic breakdown products referred to above because gastric proteolytic activity in pigs is minimal during the first 3-4 weeks of life (Cranwell & Moughan, 1989). Intact proteins have been found to be relatively ineffective as stimulators of gastrin release when placed either in an antral pouch of mature dogs or in the incubation media of cultures of isolated rodent G cells (Elwin, 1974; Lichtenberger et al. 1980). Further, the possibility that colostrum contains an inhibitor of gastrin secretion to protect the immunoglobulins from damage by gastric acid should not be discounted and warrants investigation.

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THE EFFECTS OF FEED INTAKE AND DIETARY FIBRE LEVELS ON THE ENDOGENOUS ILEAL AMINO ACID OUTPUT IN GROWING PIGS

S. Furuya and Y. Kaji

Kyushu National Agricultural Experiment Station, Nishigoshi, Kumamoto, Japan

Abstract

The effects of level of feed intake and dietary neutral detergent fibre (NDF) on the endogenous ileal output of amino acids (AA) and nitrogen (N) were studied with pigs fitted with a simple T cannula at the terminal ileum. The endogenous ileal output values of AA and N expressed as g/kg dry matter (DM) intake decreased significantly ($P<0.05$) with increasing of DM intake except for proline. In contrast, the values expressed as g/d remained a constant quantity. The effects of dietary NDF levels on the endogenous ileal output of AA and N were not significant ($P>0.05$), although the values tended to increase as dietary NDF levels increased for most AA and N.

Keywords: amino acids, endogenous output, pig.

Introduction

In the determination of the true ileal AA digestibility, it is necessary to know the endogenous ileal AA output. The most commonly used reference base for the endogenous ileal AA is DM intake, assuming that the amount of endogenous ileal AA is directly related to DM intake, but there have been no studies specifically designed to evaluate the influence of feed intake levels on the endogenous ileal AA output. Green et al. (1987) found a decrease in the apparent ileal AA digestibility with decreasing feed intake. From this finding, Green et al. (1987) concluded that there was not a direct relationship between DM intake and endogenous ileal AA output, and that endogenous output remained relatively similar at different intake levels. Although an increase in endogenous ileal AA output with higher fibre levels in the diets has been demonstrated (Sauer et al., 1977;Taverner et al., 1981), Drake (1990) found no effect for the endogenous ileal N output due to increase in dietary fibre levels. The present study was designed to determine the effect of feed intake (Experiment 1) and dietary fibre contents (Experiment 2) on the endogenous ileal flow of AA and N in pigs.

Materials and Methods

In Experiment 1, six pigs weighing approximately 45 kg initially were used in a replicated 3x3 Latin square design, involving three feed intake levels and three periods. The same pigs were used again in the same experimental design when they reached 90kg. In Experiment 2, a 5x5 Latin square design, involving five dietary fibre levels and five periods, was conducted with five pigs (approximately 32 kg initially). All the pigs were fitted with a simple T cannula at the terminal ileum. The cannula and surgical technique were identical to these described by Furuya et al. (1974).

Five protein-free diets into which purified wood cellulose was

incorporated at the expense of maize starch to provide five levels of NDF at 30 g/kg increments were used. Chromic oxide was added to each diet as an indigestible marker. In Experiment 1, the protein-free diet with 90g NDF/kg was fed at three levels, i.e. 0.8, 1.2 and 1.6 kg/d(air-dry basis). In Experiment 2, the pigs were given 1.2 kg/d of one of the protein-free diets in each period.

Each test period lasted 4 d and equal amounts of feed were given three times daily at 01.00, 09.00 and 17.00 hours. Water was supplied ad libitum. Each period of 4 d started at the 17.00 hours feeding and samples of ileal digesta were obtained between 13.00 and 15.00 hours for the three consecutive days starting 44-46 h after the initial feeding, as described in a previous paper (Furuya & Kaji, 1989).

The analysis of N, DM and crude fibre in feed and ileal digesta were carried out according to the Association of Official Analytical Chemists (1975). Dietary NDF content was determined by the method of Van Soest & Wine (1967) and chromic oxide according to the method of Fenton & Fenton (1979). The AA composition in acid hydrolysates was determined by a model LC-6A Shimadzu AA analyzer. Protein hydrolysis was carried out with 6M hydrochloric acid in sealed, evacuated tubes maintained at 110 C for 24 h. Tryptophan and cystine were not determined.

The data were analyzed by analysis of variance by the method of Snedecor & Cochran (1967). The values obtained at the different levels of feeding or dietary fibre were subjected to simple linear regression analysis. The output of AA and N through the terminal ileum were determined from the ratio of chromic oxide in the diet to that in the ileal digesta.

Results and Discussion

The endogenous ileal AA and N output, expressed as g/kg DM intake, are shown in Table 1. The data for one pig at the 1.2 kg/d level determined at the initial body weight of 90 kg were discarded as the AA and N to chromic oxide ratios of the ileal digesta sample were inordinately high (approximately twice the value of other pigs on the same level of feeding). The endogenous ileal output values decreased with the increasing level of food intake. The slopes of the regression equations of endogenous output to feeding level were significantly different ($P<0.05$) from zero for all AA and N except for proline.

The endogenous ileal AA and N output, expressed as g/d, were not affected ($P>0.05$) by the level of the protein-free diet intake (Table 2). The slopes of the regressions of endogenous output to feeding level were not significantly different ($P>0.05$) from zero for all AA and N, so mean values over all feeding levels were calculated for each initial body weight. Comparison of the endogenous output between the initial body weight of 45 and 90 kg indicated no significant differences ($P>0.05$) for arginine, leucine, lysine, methionine, alanine, aspartic acid, glutamic acid, glycine, proline and tyrosine. However, for histidine, isoleucine, phenylalanine, threonine, valine, serine and N, the daily output determined at the initial body weight of 90 kg was significantly higher ($P<0.05$) than that at the initial body weight of 45 kg.

As shown in Table 3, the endogenous ileal AA and N output were not affected ($P>0.05$) by the level of NDF intake, although values tended to increase as dietary NDF levels increased for most AA and N; the slopes of the regression equations of the endogenous ileal output to dietary NDF levels were positive for all AA and N except for arginine and proline.

The results in the present study indicated that the daily endogenous ileal output of AA and N was more or less the same, in spite of large

Table 1. Endogenous ileal output of amino acids and nitrogen (g/kg dry matter intake) at three levels of feeding determined at the initial body weight of 45 and 90 kg in pigs given a protein-free diet containing NDF 90 g/kg.

(Mean values. No. of pigs in parentheses)

	45 kg			90 kg			
Feeding level (kg/d)	0.8 (6)	1.2 (6)	1.6 (6)	0.8 (6)	1.2 (5)	1.6 (6)	Slope of regression
Indispensable							
arginine	0.98	0.68	0.52	1.14	0.80	0.75	-0.53**
histidine	0.40	0.27	0.18	0.46	0.36	0.28	-0.25***
isoleucine	0.59	0.44	0.28	0.70	0.56	0.39	-0.38***
leucine	1.10	0.73	0.50	1.16	0.94	0.66	-0.68***
lysine	1.05	0.68	0.48	0.91	0.72	0.51	-0.61***
methionine	0.24	0.14	0.12	0.28	0.19	0.13	-0.17***
phenylalanine	0.72	0.44	0.29	0.92	0.70	0.56	-0.50***
threonine	1.15	0.68	0.51	1.30	0.99	0.68	-0.79***
valine	0.91	0.54	0.39	1.02	0.79	0.55	-0.62***
Dispensable							
alanine	1.35	0.80	0.58	1.33	1.00	0.76	-0.84***
aspartic acid	1.86	1.20	0.87	1.93	1.55	1.13	-1.11***
glutamic acid	2.44	1.52	1.07	2.38	1.81	1.29	-1.54***
glycine	3.25	1.72	1.20	3.08	2.31	2.12	-1.87*
proline	5.01	4.20	3.06	6.90	4.17	5.24	-2.25NS
serine	1.21	0.76	0.57	1.43	1.10	0.80	-0.79***
tyrosine	2.09	1.31	0.96	1.69	1.52	0.91	-1.20***
Nitrogen	4.58	3.26	2.20	5.48	3.81	3.24	-2.88***

NDF, neutral detergent fibre; NS, not significant
Slope of the regression of endogenous output to feeding level
* P<0.05, ** P<0.01, *** P<0.001

variations in DM intake (Table 2). On the assumption that the endogenous output is divided into two fractions; a constant fraction and a fraction which varies directly with DM intake, the relationship between the endogenous ileal output (EI) expressed on a daily amount basis and DM intake can be expressed as follows:

EI of AA (g/d)= a + b x DM intake (kg/d) (1)

in which "a" is the constant and "b" is the slope of this equation. The endogenous output per unit of DM intake can be calculated as follows:

$$\text{EI of AA (g/kg DM intake)} = \frac{a}{\text{DM intake (kg/d)}} + b \quad (2)$$

Since the slopes of the regression equations were not significantly different (P>0.05) from zero for all AA and N in the present study, the slope constant "b" can be neglected. Assuming that the slope is negligible, the relationship between the endogenous ileal output (y, g/kg DM intake) and DM intake (x, kg/d) for lysine is expressed as an equation of "y=0.68/x" in which the constant "a", 0.68, represents the average endogenous lysine output (g/d) determined at the both initial body weights. Lysine was selected as an example because lysine is frequently the first-limiting AA in many feedstuffs. The relationship indicates that

Table 2. Endogenous ileal output of amino acids and nitrogen (g/d) at three levels of feeding determined at the initial body weight of 45 and 90 kg in pigs given a protein-free diet containing NDF 90 g/kg.
(Mean values. No. of pigs in parentheses)

	45 kg				90 kg				
Feeding level(kg/d)	0.8 (6)	1.2 (6)	1.6 (6)	overall mean	0.8 (6)	1.2 (5)	1.6 (6)	overall mean	Slope of regression
Indispensable									
arg	0.67	0.69	0.70	0.69	0.78	0.82	1.03	0.88	0.18NS
his	0.27	0.27	0.25	0.27	0.31	0.37	0.38	0.36	0.03NS
ile	0.40	0.45	0.38	0.41	0.48	0.58	0.54	0.53	0.03NS
leu	0.75	0.74	0.69	0.73	0.79	0.97	0.91	0.88	0.03NS
lys	0.71	0.70	0.65	0.69	0.62	0.73	0.69	0.68	0.01NS
met	0.16	0.14	0.16	0.15	0.19	0.19	0.17	0.18	-0.01NS
phe	0.49	0.45	0.39	0.45	0.63	0.71	0.76	0.70	0.02NS
thr	0.78	0.69	0.69	0.72	0.89	1.02	0.93	0.94	-0.03NS
val	0.62	0.55	0.53	0.57	0.70	0.81	0.75	0.75	-0.03NS
Dispensable									
ala	0.92	0.82	0.79	0.84	0.91	1.02	1.04	0.99	0.00NS
asp	1.27	1.23	1.19	1.23	1.32	1.59	1.55	1.48	0.10NS
glu	1.67	1.56	1.47	1.56	1.63	1.85	1.76	1.74	-0.04NS
gly	2.22	1.77	1.64	1.88	2.10	2.37	2.90	2.46	0.14NS
pro	3.42	4.31	4.18	3.97	4.71	4.28	7.17	5.45	2.00NS
ser	0.83	0.77	0.78	0.79	0.98	1.13	1.09	1.06	0.04NS
tyr	1.42	1.34	1.31	1.36	1.16	1.55	1.24	1.30	-0.02NS
Nitrogen	3.13	3.34	3.01	3.16	3.74	3.91	4.43	4.03	0.36NS

NDF, neutral detergent fibre; NS, not significant
Slope of the regression of endogenous output to feeding level

the endogenous ileal output of lysine expressed per unit of DM intake increases when DM intake is reduced. It is obvious that a value for the endogenous ileal AA output expressed on a DM intake basis determined with one level of DM intake should not be used in other experiments if the DM intakes differ.

Several workers (Sauer et al., 1977; Taverner et al., 1981) have reported an increase in the endogenous ileal AA output per unit of DM intake with increasing dietary fibre. However, in the present study, this effect was found not significant (P>0.05). Similar results were also found in de Lange et al. (1989) and Drake (1990) studies. In the study of Sauer et al. (1977), it was found that when pigs fed on protein-free diets containing increasing levels of cellulose (50, 100 or 150 g/kg), the total ileal AA, expressed as g/kg DM intake, was 10.95, 13.79 and 14.13 g/kg DM intake, however, when expressed in a daily bases, were 17.0, 18.7 and 15.7 g/d. This was due to the effect of dietary fibre contents to the food intake, in this study, the daily food intake were reduced (1551, 1357 and 1109 g/d) in response to the increasing levels of cellulose in the diet (Sauer et al., 1977). Taverner et al. (1981) observed significantly greater (P<0.05) endogenous ileal levels with pigs given a protein-free diet with 50 g cellulose/kg than with no added fibre, but only slight increases in the endogenous ileal output when increasing dietary NDF level from approximately 50 to 140 or to 190 g/kg; the magnitudes of the

Table 3. Endogenous ileal output of amino acids and nitrogen (g/d) in pigs given protein-free diets with different dietary NDF levels.
(Mean values of five pigs and standard errors)

	NDF level in diet (g/kg)						Slope of regression ($x10^{-3}$)
	30	60	90	120	150	sem	
Indispensable							
arginine	0.70	0.74	0.82	0.77	0.67	0.17	-0.12NS
histidine	0.29	0.26	0.30	0.30	0.32	0.04	0.35NS
isoleucine	0.40	0.36	0.42	0.41	0.46	0.05	0.58NS
leucine	0.69	0.61	0.72	0.73	0.80	0.10	1.12NS
lysine	0.53	0.49	0.56	0.56	0.62	0.06	0.81NS
methionine	0.14	0.13	0.14	0.14	0.16	0.02	0.14NS
phenylalanine	0.52	0.51	0.59	0.56	0.64	0.09	0.94NS
threonine	0.63	0.57	0.69	0.69	0.76	0.10	1.26NS
valine	0.55	0.50	0.60	0.60	0.66	0.08	1.01NS
Dispensable							
alanine	0.78	0.70	0.81	0.77	0.77	0.11	0.18NS
aspartic acid	1.12	1.06	1.16	1.15	1.16	0.15	0.60NS
glutamic acid	1.39	1.23	1.42	1.41	1.45	0.16	0.96NS
glycine	1.73	1.87	2.78	2.12	1.70	0.57	0.63NS
proline	4.31	3.66	5.44	5.15	2.80	1.52	-5.16NS
serine	0.77	0.69	0.83	0.81	0.83	0.12	0.80NS
tyrosine	1.45	1.42	1.46	1.45	1.54	0.23	0.74NS
Nitrogen	2.97	2.79	3.67	3.44	2.82	0.50	1.19NS

NDF, neutral detergent fibre; NS, not significant
Slope of the regression of endogenous output to NDF level

increases were generally similar to those observed with dietary NDF levels ranged 30 to 150 g/kg in the present study (Table 3). Furthermore, Taverner et al. (1981) reported that an increase in the amount of indigestible DM passing through the ileal caused no apparent increase in endogenous protein. From the results of the present study and others (Sauer et al., 1977; Taverner et al., 1981; de Lange et al., 1989; Drake, 1990), it may be concluded that the dietary fibre levels do not greatly affect the endogenous ileal AA output except when the dietary fibre levels are too low.

The result of the present study leads to a conclusion that the daily endogenous ileal output of AA and N remains relatively similar at different DM intake and dietary fibre levels. This conclusion is different from that made about the endogenous faecal N output (Mitchell, 1924). Mitchell (1924) reported that the endogenous faecal N of rats varied with feed intake and also with the fibre content of the diet. This has been observed with pigs (Whiting & Bezeau, 1957). The increase in the endogenous faecal N output with DM intake and dietary fibre level is probably a resultant of the action of the microflora in the large intestine, since a large proportion of faecal N from pigs to be of bacterial origin (Mason et al., 1976). The rate of fermentation, and hence bacterial protein in faeces, can be stimulated by additional substrate provided by increased DM, more specifically indigestible DM intake, as suggested by Taverner et al. (1981) and Low (1982). In contrast with the endogenous faecal N, the endogenous ileal output of AA and N is suggested

to be little influenced, if any, by the action of the microflora (Mason et al., 1976; Laplace et al., 1989).

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ENDOGENOUS N LOSSES AT THE TERMINAL ILEUM OF YOUNG PIGLETS FED DIETS BASED ON EITHER SKIMMILK POWDER OR SOYBEAN MEAL

C.A. Makkink[1], T. Heinz[2]

[1] Agricultural University, Department of Animal Nutrition, Haagsteeg 4, 6700 PM Wageningen, The Netherlands

[2] Research Centre of Animal Production Dummerstorf-Rostock, Division of Animal Nutrition 'Oskar Kellner', Rostock, Germany

Introduction

Development of surgical techniques and analytical methods enables research workers in the field of physiology to investigate in more detail the digestive processes in domestic animals. Cannulation techniques can be used to measure ileal in stead of faecal digestibility.
Until now, protein free diets and regression techniques are used to assess true compared to apparent digestibility (Souffrant, 1991).
Especially with young animals, low (protein) digestibilities are found at the ileal and the faecal level. The causes of low apparent protein digestibility can be found by comparing true to apparent digestibility. When true digestibility is also low, the feed protein itself is poorly digested, however, when true digestibility is high, poor results could be caused by an increase in endogenous N losses (e.g. through hypersecretion by the pancreas).
Recently, the 15N infusion method has been introduced (Souffrant et al., 1981, De Lange et al., 1990). With this technique it is possible to measure endogenous N losses at the terminal ileum and hence to calculate "real" digestibilities at the faecal or ileal level. The comparison of "real" with apparent digestibility yields information on the causes of low apparent protein digestibility. For a review on the 15N infusion technique and its (im)possibilities see Souffrant et al. (1982) and Souffrant (1991).
Thus, it is possible to collect more reliable data on digestibilities of various feedstuffs and on digestive processes in various animal species.
Especially on young pigs, not much is known about the causes of low (apparent) digestibilities of e.g. certain protein sources. Digestibility of soy protein is lower for piglets than for older pigs, digestibility of milk protein is high for pigs of all ages (Wilson & Leibholz, 1981a,b,c).
We used the 15N infusion technique to assess the endogenous N losses at the terminal ileum in young piglets (age 4 to 5 weeks at the start of the experiment). The milk protein diet used in this study was regarded as a control diet. Apparent digestibility of milk proteins is very high, even in young piglets, therefore, endogenous N losses were expected to be low with this diet.

Experimental Design

In this experiment 5 piglets (of ± 8 kg liveweight at the start of the experiment) were used to measure apparent and real ileal protein digestibilities of two different diets.

Composition of the diets is given in Table 1.
Piglets were fed twice daily at 8.00 h and 20.00 h. Two piglets were fed the diet based on skimmilk powder (SMP) and three piglets were fed the diet based on soybean meal (SBM). The diets were fed at a level of 380 g/day throughout the experiment. After a 6 day "cage adaptation" period, the piglets were fitted with a PVTC cannula in the caecum (Van Leeuwen et al., 1990). After surgery, a 7 days recovery period was allowed before blood vessel catheterization: catheters were placed in the jugular external vein (for continuous 15N infusion) and in the carotid artery (blood sample collection). After catheterization, another 4 days recovery period followed before 15N infusion started. The experimental scheme is presented in Figure 1.
The continuous intravenous 15N-L-Leucine infusion was performed at a rate of ± 40 mg 15N-L-Leucine (95% 15N enrichment) per kg body weight per day.
From the start of 15N infusion faces was collected continuously for 6 days. During this period the PVTC cannula was closed. Apparent faecal digestibility is determined from the ingested feed and the excreted faeces during these 6 days. At day 7, 9 and 11 after the start of 15N infusion, ileal chyme was collected for 24 hours a day. Digesta was collected, weighed and frozen hourly, pooled per animal per day. Apparent ileal digestibility is determined from the ingested feed and the collected chyme at these 3 days.
Blood samples were taken twice daily from the carotis catheter during feeding at 8.00 h and 20.00 h. Blood plasma protein was precipitated using 20% trichloroacetic acid and N and 15N was analyzed in the supernatant (TCA soluble fraction) and the precipitate (TCA precipitable fraction).

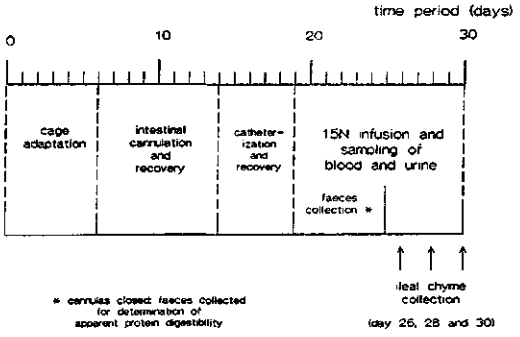

Figure 1.
Experimental scheme.

The contribution of endogenous to total N in ileal chyme or faeces can be calculated from the ratio of 15N enrichment excess in ileal chyme or faeces and in the blood TCA soluble fraction, assuming that the 15N excess in the endogenous N and in the blood TCA soluble fraction is similar. The calculations are carried out according to Souffrant et al. (1986) using the following equation:

$$Nen = Ntot * \frac{Nex\ c/f}{Nex\ bl}$$

Ntot =total N (g/day)
Nen=endogenous N (g/day)
Nex = 15N excess
c=chyme f=faeces bl=blood

Table 1. Composition of the diets

ingredient	diet SMP	diet SBM
soybean meal (47.5 % CP)	-	34.40
skimmilk powder (35.3 % CP)	45.50	-
maize starch	29.60	39.84
dextrose	15.00	15.00
sunflower/soy oil	2.00	2.00
cellulose	5.00	2.85
vit/min premix	1.00	1.00
ground limestone	0.80	1.35
mono Ca phosphate	0.50	2.10
salt	-	0.50
$KHCO_3$	0.10	-
$NaHCO_3$	0.30	0.40
L-Lysine HCl	-	0.16
DL-Methionine	0.10	0.20
L-Threonine	-	0.10
Cr_2O_3	0.1	0.1
calculated contents		
crude protein	16.08	16.37
digestible CP	15.44	14.76
net energy (kcal/kg)	2555	2477
crude fat	2.49	2.44
crude fiber	4.95	5.02
ash	5.33	5.88

Table 2. Results of ^{15}N infusion experiment

diet	SMP skimmilk powder	SBM soybean meal
number of piglets	2	3
apparent ileal N digestibility (%)	84.4 ± 0.1	76.5 ± 2.6
endogenous ileal N mg/day	786 ± 42	1423 ± 372
endogenous ileal CP g/100 g DM intake	1.41 ± 0.78	2.61 ± 0.68
endogenous ileal CP g/100 g CP intake	8.3 ± 0.4	14.1 ± 3.7
endogenous ileal CP mg/kg LW	435 ± 23	829 ± 218
true ileal N digestibility (%)	92.7 ± 0.0	90.6 ± 1.1

True protein digestibilities can be calculated from the apparent digestibilities by subtracting the endogenous protein (Huisman, 1990).

Results

The results of the trial are given in Table 2. Apparent ileal N digestibility was higher with the SMP diet than with the SBM diet (84.4 vs 76.5 %). Endogenous N losses at the terminal ileum were ± twice as high with the SBM diet, resulting in almost similar true ileal N digestibilities for the two diets (92.7 and 90.6 % for the SMP and the SBM diet resp.). From these data it is concluded that the differences in apparent ileal N digestibility between the two diets are not caused by differences in the (true) digestibilities of the two protein sources. The SBM diet induced an increase in endogenous N secretion, resulting in a decrease in apparent N digestibility.

Discussion

Wilson & Leibholz (1981c) studied apparent and true N digestibilities of diets containing milk or soybean protein with piglets (28 days of age) using a nitrogen free diet. For the milk protein diet they found apparent and true ileal N digestibilities of 86 and 92% respectively, which closely resembles our values of 84 and 93% respectively. Apparent protein digestibility was expected to be higher for the milk protein diet because of the adaptational status of the newly weaned pig. The relatively low digestibility could be due to differences between cow's milk protein and sow's milk protein. Wilson & Leibholz (1981c) reported apparent and true ileal N digestibilities for the soybean meal diet of 51 and 62% resp. In our study we found apparent and true ileal N digestibilities for the SBM diet to be 77 and 91% respectively. This difference could be due to age of piglets (in our study 35 - 50 days) or to diet composition (Wilson & Leibholz (1981c) used diets containing ± 27% of crude protein) or to an interaction between age and diet composition. The latter option has been supported by Wilson & Leibholz (1981a) who studied performance of piglets given milk and soybean proteins at different ages. They found an interaction between protein source in the feed and age of the piglets: performance of piglets fed milk protein did not change between 7 and 35 days of age, however, performance of piglets fed soybean protein did increase with increasing age.
Wilson and Leibholz (1981c) studied endogenous N flow at different sites of the gastrointestinal tract of piglets (35 days of age) fed a protein free diet. They reported an endogenous N flow at the terminal ileum of 0.82 g N per day. In our study we found N flows of 0.79 and 1.42 g N per day for piglets fed the SMP diet and the SBM diet respectively. These data indicate that endogenous losses are minimal when milk proteins are fed to young piglets.
However, endogenous N losses measured with animals fed protein free diets possibly do not reflect the normal, physiological situation when protein containing diets are fed. The fact

that different protein sources in the feed induce different levels of endogenous N secretion stress this aspect (Makkink et al., in preparation).

Leibholz (1982) studied the endogenous nitrogen secretion in young pigs (4 weeks of age). She used the regression method to assess endogenous N losses with a feed based on milk protein. By means of regression analysis she calculated endogenous N flow at the terminal ileum to be 3.44 g N per kg dry matter intake, which amounts to 2.2 g endogenous ileal crude protein per 100 g dry matter intake. From our study we calculated 1.41 and 2.61 g endogenous ileal crude protein per 100 g dry matter intake for the SMP and the SBM diet resp.. From our experiment we concluded that true ileal N digestibility of soybean meal is not much different from true ileal N digestibility of skimmilk powder in young piglets. Therefore, differences between these two protein sources with regard to apparent ileal N digestibility and performance of piglets (growth, feed conversion ratio) must be caused by an increase in endogenous N secretion with piglets fed soybean meal. The soy protein itself was highly digestible (91% at the ileal level), but it probably stimulated nitrogen secretion by the exocrine glands of the digestive tract, resulting in endogenous N losses.

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ENDOGENOUS LOSSES OF PROTEIN FROM HEAT PROCESSED BEANS (PHASEOLUS VULGARIS L.)

A.F.B. van der Poel[1], Th. Heinz[2], M.W.A. Verstegen[1], J. Huisman[3] and W.B. Souffrant[2]

[1] Agricultural University, Department of Animal Nutrition, Haagsteeg 4, 6700 PM Wageningen, The Netherlands

[2] Research Centre of Animal Production Dummerstorf-Rostock, Division of Animal Nutrition 'Oskar Kellner', Rostock, Germany

[3] TNO-Institute of Animal Nutrition and Physiology, P.O. Box 15, 6700 AA Wageningen, The Netherlands

Abstract

The present investigation was undertaken to study the apparent and true ileal digestibility of steam processed common beans (Phaseolus vulgaris L.) in piglets. The ^{15}N dilution technique was used. The apparent ileal protein digestibility of common beans, steam processed at 102 °C during 40 min, was about zero while the true ileal digestibility was about 65%. The apparent ileal digestibility of dry matter (DM) was about 70%.

From our studies we concluded that even after correction for endogenous protein the true digestibility of common beans is low. This means that the storage proteins in beans are resistant to enzymatic digestion after heating at certain conditions. The results of the present investigation can explain the reduced weight gain of piglets fed diets with heated Phaseolus beans from previous experiments. In addition, the commonly applied heating procedures for Phaseolus beans (heating at 100 °C) are insufficient to guarantee a high true protein digestibility.

Introduction

Raw common beans (Phaseolus vulgaris L.) have a relatively low feeding value owing to the lectin proteins and to trypsin inhibitors present in the cotyledons. These so-called anti-nutritional factors (ANF) reduce the biological availability and digestibility of one or more nutrients (Sgarbieri and Whitaker, 1982). Technological treatment like steam processing may eliminate or reduce the ANF contents to very low levels. Steam processed beans, however, still have a low feeding value based on apparent faecal (Rodriguez & Bayley, 1987) or ileal (Huisman et al., 1991; Van der Poel et al., 1991a) digestibility values. This feeding value is still lower than can be expected on the basis of the analysed residual ANFs. Therefore, it is needed to study the effect of thermal processing of beans in more detail and to correct the apparent protein digestibility for the excretion of endogenous protein in order to obtain true digestibilities. In this way it is possible to distinguish between the effects of processing on the resistance of bean storage protein to enzymatic digestion and the effects of endogenous proteins as related to residual (low) levels of ANF, particularly lectins (Kik et al., 1989).

The aim of the present investigation was to measure the true digestibility of DM and protein of common beans, steam heated at 102 °C during 40 min using the ^{15}N dilution technique. With this technique, the body protein which constitutes the excreted endogenous protein and which are lost to the animal, is labeled and then measured in the ileal chyme (Souffrant et al., 1986). In this way, one can differentiate the total apparently ingested protein between the excreted non-digested dietary and excreted non-absorbed endogenous protein.

Materials and Methods

A batch of common beans (Phaseolus vulgaris L., cull grade) was characterized for physical properties and chemical composition. Then they were steam processed as described previously in detail (Van der Poel et al., 1990). A laboratory-scale toaster was used. After heating, the bean samples were air-dried (35 °C, 24 h) prior to milling (stepwise: 6 and 1 mm, respectively), storage at 4 °C and analyses.

In order to establish that the bean protein was the only protein source in the diet, beans were fine milled (pin mill) and air classified (Alpine 132 MP classifier) to obtain a common bean protein concentrate.

From a preliminary experiment with intact piglets it was derived, that the diet containing protein that originated only from Phaseolus beans was not digested very well. Only eight hours after the first feed intake, dirty, yellow-coloured diarrhoea was observed while a control group of piglets showed normal faeces. The Phaseolus diet (PH), therefore, was mixed with a diet containing soyabean isolate (SI) used in a previous ^{15}N experiment (Makkink et al., 1991; In preparation). This diet was checked for feed acceptance first. In general, the feed acceptance for the 60% SI/40% PH mixture was sufficient and was used now in the present investigation.

In the ^{15}N experiment, treatment groups comprised of 4 (SI/PH) and 5 (SI) piglets. They were surgically fitted with a PVCT canula in the ceacum after an adaptation period of 6 days to the diet. After a period of 7 days to allow for recovery, the piglets were provided with a cathether in the jugular external vein and carotic artery for the continuous infusion of a ^{15}N-L-leucine solution and for blood collection, respectively. Recovery was about 5 days.

Continuous intravenous infusion of ^{15}N-L-leucine was performed at a rate of about 40 mg ^{15}N-L-leucine (95% ^{15}N enrichment) per kg bodyweight per day during 11 days. The contribution of endogenous to toal N in ileal chyme can be calculated from the ratio of ^{15}N enrichement excess in ileal chyme and in the blood TCA soluble fraction, assuming that the ^{15}N excess in the endogenous N and the blood TCA soluble fraction is similar. The calculations were carried out as described by Souffrant et al. (1986).

Ileal chyme was collected for 24 hours at three separate days; days 7,9 and 11 after the start of the infusion period. The digesta samples were pooled per animal per day. The chyme was collected in small plastic bags attached to the canula. Each hour the flow into bags and the attachement were controlled. When chymus was produced the bag was weighed and immediately frozen at -20°C. Blood samples (10 ml) were taken twice daily from the carotic artery catheter during feeding at 8.00 and 20.00 h. The true protein digestibilities then were calculated from the apparent ileal digestibilities by correcting for the contribution of endogenous protein. The digestibility of N and DM of the Phaseolus beans was calculated then by difference.

Results

The effects of steam processing (Table 1) clearly indicate a reduced level of proteinaceous ANF and paralleled the decrease in the dispersibility of the protein. The total lectin content (measured by ELISA) was reduced to about 5% of the level of the untreated beans.

Table 1. Effect of steam processing on lectins[a], trypsin inhibitor activity (TIA) and protein dispersibility index.

Criterium	Raw beans	Heated beans
PDI (%)	34.2	18.2
TIA (mg g^{-1})	10.13	0.94
Lectins		
- HA (units g^{-1})	64	20
- ELISA (mg g^{-1})	58.0	3.1

[a] HA = haemagglutination activity

In Tables 2 and 3, the main diet ingredients and the chemical composition of diets are shown, respectively.

Table 2. Main ingredients and composition (g kg^{-1}) of the diets[a,b].

Ingredient	SI/PH diet	PH diet
Soya isolate	109	-
Phaseolus, toasted	87	217
Phaseolus, air classified[c]	71	177
Corn starch	416	249
Dextrose	194	261
Sunflower oil	19	17
Cellulose	42	30
Vit/min mixture	10	10

[a] Further ingredients: NaCl, $NaHCO_3$, $KHCO_3$, monocalciumphosphate, limestone, Dl-methionine, L-threonine, L-tryptofaan, L-lysine-HCl, chomic oxide.

[b] SI, soyabean isolate; PH, Phaseolus vulgaris

[c] Air classification after toasting

Table 3. Analyzed composition (g kg^{-1}) of diets.

	SI/PH diet	PH diet
Dry matter	900	889
Ash	48	46
Crude protein	152	141
Crude fat	21	28
Crude fibre	38	38

The replacement of about 37% of the SI protein by Phaseolus protein showed a clear decrease of the apparent digestibility of protein (SI/PH diet; Table 4). This reduction was noticed regardless to the fact that ANF had been reduced to very low levels. The calculation of the ileal digestibility of Phaseolus protein of the PH-diet therefore showed low values, and sometimes they were even negative. The digestibility of DM decreased from 83% (SI diet) to 69% (SI/PH diet) to about 45% (PH diet; difference method). The lower DM% of chyme (7.4% vs 11.7%) suggest disturbed digestion processes. It will be interesting to know what physiological processes are responsible for this.

Table 4. Apparent and true ileal DM and protein digestibility (%) of Phaseolus vulgaris beans and excretion of endogenous protein (Difference method)[1]

	SI/PH diet		PH diet	
	Protein	DM	Protein	DM
Endogenous secretion				
g 100 g^{-1} DM intake	6.6 ± 2.7		10.7 ± 6.8	
g 100 g^{-1} protein intake	38.9 ± 15.8		67.6 ± 42.6	
Apparent ileal digestibility	47.5 ± 12.4	69.6 ± 3.7	-3.9^{a} ± 33.3	44.0 ± 12.5
True ileal digestibility	86.3 ± 4.1	-	65.8^{b} ± 11.3	-

[1] Mean and standard deviation (n=3)

[a,b] Values with different superscript differ significantly ($P<0.05$)

Discussion

The endogenous quantity of N shows that in the chyme of piglets an essential part of the chymus-N quantity is of endogenous origin. Although only 37% of the protein in the SI/PH diet originates from Phaseolus beans, it can be calculated that for the 3 piglets 51, 66 and 77%

of the total endogenous N-secretion, respectively, can be attributed to the PH diet. The standard deviation of the particular parameteres of the endogenous ileal N secretion were 2 to 3 times higher compared to the control group. The large variation between the animals suggests that there may be distinct variation in individual tolerance of ANF from Phaseolus beans.
The true N digestibility of Phaseolus bean proteins was about 66% (Table 4) which is considered rather low. The very low even negative apparent digestibility of N shows that more than 65 g of endogenous protein per 100 g of protein passes the distal ileum. Probably, the bean proteins are difficult to digest on the basis of their structure. This structure may have been affected by the thermal treatment. A further possibility is that the interaction of residual lectins (Table 1) with small intestinal epithelium has caused intestinal disturbance during the 3-weeks feeding period before the digesta collection was started. The absorption of end products from digestion then, may have been negatively influenced (Kik et al., 1989). Therefore, the SI and SI/PH diets were studied on their effects on the morphology of cultured explants of the small intestinal mucosa according to Kik et al. (1988). The results are shown in Table 5.

Table 5. Morphometric variables of pig jejunal explants of the SI and SI/PH diet.

	Villus length (V) (μm)	Crypt depth (C) (μm)	Ratio (V/C)
SI diet (control)	382	268	1.4
SI/PH diet	290	312	0.9
Optimum values[a]	500-600	100-200	2.5-3.5

[a] M.J.L. Kik, 1990, pers. communications

Finally, it needs to be evaluated whether the storage protein of bean (Glycoprotein II in particular) may stimulate the secretion of endogenous protein in the small intestine as suggested by Santoro et al. (1989). Although the control animals show no optimal value for villus length and crypt depth, the morphometric values of the animals on the SI/PH diet are much worse. The largest damage was observed for the piglet of which explant showed a villus length of 220 μm, a crypt depth of 370 μm and a V/C ratio of 0.6. These results suggest negative influence on the potential intestinal absorption of nutrients.
The present investigation show that the protein of Phaseolus vulgaris beans, steam heated at 100°C during 40 minutes, is highly resistant to enzymatic digestion. It may be that the heating time of 40 min at atmospheric heating is too long and may have induced this resistancy. In a 'mobile nylon bag' digestibility trial (Van der Poel et al., 1991b) it was found that steaming of beans at 140°C during 1.5 min showed a higher ileal digestibility of protein compared to atmospheric steaming during longer duration. These results give some indication that the true digestibility of Phaseolus beans is dependant on the temperature/time relationship during heat processing.

In conclusion, the low apparent ileal protein digestibility is related to a large extent to the excretion of endogenous protein. Still, more detailed information is needed on the effect of low levels of lectin proteins on the interaction with intestinal absorption. In view of the efficiency of the various heating procedures employed in the animal feed industry, the effect of bean storage protein (Glycoprotein II) after heating at different conditions of intensity on the efficiency of the digestion processes and on endogenous secretion has to be elucidated.

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EFFECT OF PEAS AND PEA ISOLATES ON PROTEASE ACTIVITIES IN PANCREATIC TISSUE OF PIGLETS

M.P. Le Guen[1], J. Huisman[2] & C.A. Makkink[3]

[1] G.I.E. EURETEC, 12 avenue George V, 75008 Paris, France
[2] TNO-Institute of Animal Nutrition and Physiology (ILOB), PO Box 15, 6700 AA Wageningen, The Netherlands
[3] Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands

Abstract

Two trials with piglets were performed to study pancreatic enzyme activities in response to different diets. Piglets of 10 to 15 kg live weight were fitted with ileal cannulas to collect ileal digesta. The piglets were fed isonitrogenous diets based on casein and fishmeal (*control* diet), raw pea and air-classified pea (*RPAP*), pea protein isolate (*PPI*) or pea protein isolate enriched with pea ANF concentrate (*PPI-ANF*).
At the end of the collection periods, all piglets were killed and the pancreases removed for trypsin and chymotrypsin determinations. Results showed that inclusion of raw peas in piglets diets lowered weight gain and protease activities in pancreatic tissue. ANF concentrates added to PPI diet lowered also weight gain but not protease activity in the pancreas.
Keywords: piglet, pea, trypsin inhibitor, pancreas, trypsin, chymotrypsin.

Introduction

The inclusion of high levels of raw pea (> 15% for winter peas) in young pig feeds leads to low performances (Quemere et al. 1982; Fekete et al. 1984; Gatel et al. 1989) and to low apparent ileal digestibilities of nitrogen and amino acids (Huisman et al. 1990*a*). Antinutritional factors (ANF) - mainly trypsin inhibitors and lectins - are considered partly responsible for these observed negative effects of raw peas (Huisman et al. 1990*b*). Numerous studies dealing with several animal species (Yen et al. 1977; Roy & Schneeman, 1981; Naim et al. 1982; Hasdai et al. 1989, Huisman & Van der Poel, 1989; etc.) showed that raw soybean meal affect the pancreas. These effects would be largely associated with the content of trypsin inhibitors (Liener & Kakade, 1980). As hardly any work has been done in this respect with peas and piglets, two trials were performed to measure ileal digestibility and to assess protease activity in pancreatic tissue. Effects were measured in piglets fed raw peas and different pea fractions. Only the results concerning the pancreas are reported here, the digestibility coefficients being reported in Huisman et al. (1990*a*,*b*).

Materials and Methods

In trials 1 and 2, 15 and 10 piglets were used respectively, each fitted with a cannula at the terminal ileum to collect ileal digesta. The different diets (see Table 1) were allocated at random according to live weight and litter. After a period of digesta collection (22 and 12 days for trial 1 and 2 respectively), the piglets were killed and the pancreatic tissue was collected for enzyme analysis. Trypsin and chymotrypsin were measured according to Bergmeyer (1974), after activation of the zymogen.

Table 1. Composition (%) of the diets fed to piglets in trials 1, 2.

Trial ...	1	1	1	2	2
Diet ...	control	RPAP	PPI	PPI	PPI-ANF
Fish meal	6.9	-	-	-	-
Casein	12.5	-	-	-	-
Whole winter peas[a]	-	25.0	-	-	-
Air-classified winter pea[a]	-	17.8	-	-	-
Winter pea protein isolate[a]	-	-	17.9	-	-
Spring pea protein isolate[a]	-	-	-	18.4	16.4
ANF concentrate[b]	-	-	-	-	2.9
Maize starch	51.8	30.7	52.7	52.2	51.3
Dextrose	15.0	15.0	15.0	15.0	15.0
Sunflower oil	2.0	2.0	2.0	2.0	2.0
Cellulose	5.0	3.0	4.8	4.8	4.8
Mineral mix[a]	6.6	5.9	6.8	6.8	6.8
DL-methionine	.06	.29	.38	.37	.37
L-threonine	-	.06	.16	.14	.14
L-tryptophan	-	.06	.06	.06	.06
Chemical composition (measured)					
Crude protein (%)	16.3	16.1	16.5	17.4	17.7
TIA[c]	nd	1.89	.36	.12	1.24
Lectins[d]	nd	1915	501	507	2732

nd, not determined.

[a] For more details, see Huisman et al. (1990a,b).

[b] Mixture of ANF concentrates of the spring (Finale) and the winter (Frijaune) pea, 51 and 49% respectively.

[c] TIA, trypsin inhibitor activity; in mg inhibited trypsin/g feed, measured according to Van Oort et al. (1989).

[d] ELISA: µg lectins/g.

Results

Results of trials 1 and 2 are presented in Table 2. Initial live weights of piglets were 12.5 kg for both trials. In trial 1 the lowest daily weight gain was obtained with the piglets fed the *RPAP* diet (239g/d) compared to 323 and 296 g/d for the *control* and the *PPI* diets respectively ($p < 0.05$). In trial 2, the addition of pea ANF concentrates resulted in a lower weight gain: 229 g/d with *PPI-ANF* diet as compared to 277 g/d with *PPI* diet ($p < 0.05$).

In both trials, weight of the pancreatic tissue was not significantly affected by diet composition. The pancreas weights were 1.73, 1.56, 1.86, 1.65 and 1.62 g/kg BW for the *control*-1, the *RPAP*-1, the *PPI*-1, the *PPI*-2 and the *PPI-ANF*-2 diets respectively.

Total activities of trypsin (T) and chymotrypsin (CT) in pancreatic tissue were significantly reduced when raw peas were included in the diet. T and CT activities (10^3 Units) were respectively: 100.3 and 8.4 for the *control* diet, 34.0 and 3.7 for the *RPAP* diet, 81.7 and 7.3 for the *PPI* diet. The addition of ANF concentrates to the *PPI* diet did not affect protease activity in the pancreas (Table 2).

Table 2. Dietary effects on pancreas weight and protease activities in the pancreatic tissue of piglets (Mean values with their standard errors).

Trial ... Diet...	1 control	1 RPAP	1 PPI	2 PPI	2 PPI-ANF
Pancreas weight (g/kg BW)	1.73^{a} (.3)	1.56^{a} (.4)	1.86^{a} (.3)	1.65^{a} (.2)	1.62^{a} (.3)
Total activity in the whole pancreas (10^3 Units)					
trypsin	100.3^{a} (51.2)	34.0^{b} (16.4)	81.7^{a} (9.8)	68.3^{a} (15.5)	59.5^{a} (12.1)
chymotrypsin	8.4^{a} (4.5)	3.7^{b} (1.7)	7.3^{a} (1.4)	9.7^{a} (3.7)	8.6^{a} (2.6)

$^{a, b}$ Mean values in a row or within a trial bearing different superscripts differ significantly ($P < 0.05$).

Discussion

Weight gain of the piglets fed raw peas in this study was depressed by 24% compared to weight gain of piglets fed the *control* diet. This effect is commonly observed in growth trials with piglets (Fekete et al. 1984; Bengala Freire et al. 1989). Part of the depression seems to be related to the presence of ANF: when the ANF concentrates were incorporated in the *PPI* diet, daily weight gain was decreased by 17%.

Pancreas weight stayed unchanged when the raw pea diet was fed, in agreement with Huisman et al. (1990*c*)

The response of the exocrine pancreas of piglets fed pea ANF seems to depend on the protein source associated with them. With the *RPAP* diet, protease activities in pancreatic tissue were lower than when the diet was based on pea protein isolate. A decrease in protease activity in the pancreas has also been observed in other species when raw soybean protein was fed. This was found by Yen et al. (1977) with piglets fed raw soybeans, by Roy and Schneeman (1981) with mice fed soy protein isolate (decline of the chymotrypsin activity in that experiment), by Hasdai et al. (1989) with guinea pigs fed raw soybeans and by Khorasani et al. (1989) with veal calves fed soy protein isolate.

When the pea ANF are isolated and added to a pea protein isolate, they have no effect on the enzyme activity in the exocrine pancreas. Some caution is however required in interpreting the results. In trial 2 the pancreases were removed 14 hours after the last meal, compared to 3 hours in trial 1. According to a study of Gertler & Nitsan (1970) on chicks, starvation would have effects on pancreatic enzyme activity depending on diet composition.

The availability of amino acids may have had an important role to play in the response of the pancreas. The ileal apparent digestibilities of nitrogen and amino acids were much lower for the *RPAP* diet than for the *PPI* diet (Huisman et al. 1990*a*). With the *RPAP* diet, the supply of amino acids may have been too low to allow the pancreas for a normal synthesis of zymogens. With the *PPI* diet however, enough amino acids would have been provided for normal activities. Johnson et al. (1977) showed the importance of protein quality on the exocrine pancreas of rats; feeding a poor quality protein - zein - resulted in lower chymotrypsinogen synthesis, compared to casein.

Protease activity in the pancreas at a certain time is the resultant of synthesis and excretion of enzymes. A lower activity could then be due to the following reasons:

- a higher export of enzymes from the acinar cells to the intestinal lumen, and/or
- a lower synthesis of zymogen in the pancreas.

It is generally believed that dietary trypsin inhibitors in the intestinal lumen activate the release of cholecystokinin, a gastrointestinal hormone that would stimulate the pancreas to secrete more enzymes (Khayambashi & Lyman, 1969; Corring, 1974). If this mechanism occured in both trials, the higher export of enzymes from the pancreas would have been compensated by a higher synthesis only when the pea ANF were in the form of concentrate and not in the form of raw peas.

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SESSION 3
In vitro techniques of measuring digestion

IN VITRO TECHNIQUES OF MEASURING DIGESTION

B.O. Eggum and S. Boisen

National Institute of Animal Science, Foulum, Denmark

Abstract

Several enzymatic methods have been developed to simulate the physiological process of digestion in vivo. In general, the in vitro methods vary according to the enzymes employed, temperature and time of the reaction and the separation techniques used to obtain the digested fraction. Protein digestibility is usually measured from the quantity of nitrogen or amino acids released by the enzymatic hydrolysis of protein sources. The more appropriate approach would be to use a two-step enzymatic system consisting of first digestion with pepsin in acid medium and subsequent enzymatic hydrolysis with pancreatin in alkaline medium. In such experiments, the choice of temperature, pH, enzyme-substrate ratio, and duration of the reaction becomes crusial. In recent years, in vitro methods for the evaluation of feeds for single-stomached animals have been developed using either contents of the pig stomach and different parts of the small and large intestine or blends of pepsin and hydrochloric acid in combination with pig intestinal fluid as inocula for incubations. These methods have been shown to be well correlated with in vivo apparent faecal digestibilities for dry matter and energy.

There is a need to further develop and standardize the in vitro methods, especially those for estimating digestibility of amino acids. Moreover, interlaboratory testing and validation of the in vitro methods by in vivo methods are required before the in vitro methods can be recommended for regulatory purposes.

Key words: single-stomached animals, pepsin, pancreatin, intestinal fluid, protein, nitrogen, amino acids, starch.

Introduction

Conventional digestibility techniques involving either total faecal collection or the use of an indicator are expensive and time consuming and require relatively large amounts of feed. As a consequence, these techniques are impractical for routine feed analysis, particularly when large numbers of samples are involved. Therefore, efforts have been made to develop simple and quick laboratory methods as alternatives to in vivo trials.

In vitro techniques with rumen fluid or substitutes have been routinously and extensively used for the evaluation of ruminant feed. In recent years, in vitro methods for the evaluation of feeds for single-stomached animals have been developed using either contents of the pig stomach and different parts of the small and large intestine (Vervaeke et al., 1979; Holzgraefe et al., 1985) or blends of pepsin and hydrochloric acid in combination with pig intestinal fluid (Furuya et al., 1979; Clunies & Leeson, 1984) as inocula for incubations. These methods have been shown to be well correlated with in vivo apparent faecal digestibilities for dry matter and energy. The estimation of protein digestibility for monogastrics by in vitro procedures based on

consecutive incubation with commercial enzyme preparations has been described by several authors (e.g. Metz & Van der Meer, 1985; Babinszky et al., 1990).

The purpose of the present review is to describe and discuss current in vitro methods to predict protein and dry matter/energy digestibility in feedstuffs.

According to the different principles and equipments, the in vitro methods can be divided into three major groups based on:

A. Dialysis of digested matter during incubation

B. pH-changes during incubation.

C. Undigested matter after incubation in a closed system.

In vitro techniques

A. Dialysis of digested matter during incubation

A close simulation of the digestion processes in vivo can be obtained in vitro when the digested matter is removed continously. In this way, accumulation of end products which may reduce the enzymatic reaction (Robbin, 1978) is avoided. To overcome this problem, Mauron et al. (1955) carried out the digestion inside a dialysis bag. Based on this idea, Steinhart & Kirchgessner (1973) developed a technique including a digestion apparatus for the enzymatic in vitro digestion of proteins. The apparatus consisted of a dialysis tube that was utilized as reaction vessel and a dialysis vessel. Temperature was regulated thermostatically and pH was kept constant by means of "pH-stat" devices. The reaction mixture in the dialysis tube and the dialysis vessel was stirred continously during incubation. Model tests were conducted using soya protein as substrate and pepsin as enzyme.

This method was further developed by including a two-step proteolysis using pepsin digestion at pH 1.9 in a beaker followed by pancreatin digestion at pH 8 for 24 hours inside a dialysis bag with a molecular weight cutoff of 1000 (Gauthier et al. 1986). The dialysis bag was suspended in a buffer which could be changed continously or discontinously to remove the digested matter. The investigation of three protein sources, i.e., casein, soybean, and rapeseed proteins showed that the degree of digestion (hydrolyzed N) was markedly improved by buffer replacements, indicating inhibition from end-products. The deleterious effect of heat and alkali treatment on protein digestion was clearly demonstrated by this procedure. The mixture of amino acids and small peptides released at different rates did reflect enzyme specificity in the above mentioned three protein sources (Vachon et al., 1983).

Savoie & Gauthier (1986) improved the design of the apparatus to a completely closed system, a "digestion cell" which gave better performance and reproducibility, and optimal diffusion rate of proteolysis products during digestion was obtained. Gauthier et al. (1986) studied the optimal enzymatic conditions using casein as a reference substrate. The peptic digestion was performed with a pepsin source of high specific activity with an enzyme:substrate (E:S) ratio of 1:250. The proteolysis with pancreatin was carried out for 6 hours at an E:S ratio of 1:25. A 10 mM sodium phosphate buffer, pH 7.5 was used as the circulating dialysis buffer.

The described method can be used for direct measurement of amino acid susceptibility to hydrolysis by proteolytic enzymes. Thus, Brulé & Savoie (1988) demonstrated that basic and aromatic amino acids were generally the most readily liberated, while cystine and proline were the least digested in six common protein sources (Table 1).

Table 1. Amino acid digestibility (%) of protein sources after 6 hours of incubation (Brüle & Savoie, 1988)

Amino acids	Casein	Field peas	Peanut meal	Rapeseed	Soya bean	Wheat flour	Overall mean ± SEM
Arg	74.7	70.0	52.7	73.4	59.6	77.2	68.4±3.88
His	44.4	40.2	40.8	46.1	34.4	46.4	41.7±2.11
Ile	34.4	45.0	54.6	44.9	42.7	50.6	45.5±2.30
Leu	54.0	47.8	52.3	46.1	51.0	43.6	48.8±1.86
Lys	63.9	56.7	45.1	64.2	51.7	68.0	58.9±3.53
Met	42.3	29.7	36.4	39.7	40.3	25.3	34.8±2.83
Cys	22.4	28.9	27.7	22.1	19.8	13.4	18.8±1.75
Phe	53.5	52.3	61.4	50.1	60.4	33.0	52.2±2.09
Tyr	64.3	57.5	75.6	62.2	66.8	48.8	63.1±4.00
Thr	38.6	33.0	40.0	34.1	35.2	36.3	36.7±2.67
Val	35.7	44.6	59.8	46.5	48.6	42.1	46.6±2.98
Ala	39.8	35.8	41.2	41.3	40.7	37.2	38.7±2.52
Asp	28.2	31.0	30.9	31.7	29.2	32.0	31.0±2.06
Glu	32.4	27.3	29.7	32.1	27.3	24.7	29.0±1.81
Gly	39.8	28.9	30.9	32.9	32.8	25.3	32.0±2.09
Pro	28.6	23.7	26.9	18.8	22.5	13.4	22.1±2.28
Ser	30.7	35.0	34.8	35.7	37.1	30.5	34.1±2.13

All values are the mean of five determinations.

However, the kinetics in the hydrolysis of peptide bonds and release of amino acids and peptides are dependent on the enzyme mixture due to the different specificities of the enzymes involved. Thus, Savoie & Charbonneau (1990), investigated the effects of adding pure trypsin and chymotrypsin to pancreatin, demonstrated that the enzyme mixture required for the most rapid hydrolysis was different from one substrate to another.

In vitro and in vivo digestibilities of individual amino acids in 19 selected food products were compared on the basis of their relative digestibility index (RDI) = ratio between digestibility of a given amino acid and nitrogen digestibility (Sarwar et al., 1989). However, only a poor relationship was obtained. The authors assumed that, unlike the in vivo values, the in vitro values are based on partial digestion simulating early release of amino acids (and small peptides) in the intestinal lumen and do not include corrections for amino acids of metabolic origin (Sarwar et al., 1989). Galibois et al. (1989) compared the essential amino acid (EAA) profiles in digesta collected at 3 hour intervals during 24 hours in vitro enzymatic proteolysis of casein and rapeseed protein, with the pattern of appearance of dietary EAA in the portal vein of pigs fed the same proteins. The EAA were determined each hour over an 8 hour postprandial period by coupling blood flow rate with porto-arterial differences in plasma EAA concentrations. The results indicated a close relationship between the in vitro release of amino acids and the appearance of dietary essential amino acids in the portal

vein of the pigs. In conclusion, this method seems to be valuable when studying the luminal protein degradation and is also useful for the study and prediction of availability of dietary amino acids.

The method can be used to measure the enzymatic release of any hydrosoluble low molecular weight product from a high molecular weight substrate. This method can thus be used for the measurement of starch digestion as reported by Drake (1991).

B. pH-changes during incubation

During proteolysis, protons are released from the cleaved peptide bonds resulting in a decrease in pH in a suspension. The initial rate of proton release is believed to correlate with protein digestibility. The principle was introduced by Hsu et al. (1977). Samples of equal N-content were incubated with an enzyme mixture, consisting of trypsin, chymotrypsin and an intestinal peptidase. pH was adjusted to 8.0 and after 10 min. the pH-drop was measured and correlated to in vivo faecal apparent digestibility of protein. Satterlee et al. (1980) supplied this 3-enzyme method with a further incubation comprising a microbial protease from Streptomyces griseus (4-enzyme method). The validity of these metods was investigated by Pedersen & Eggum (1981) and Wolzak et al. (1981). Both groups recommended it necessary to use different equations for each class of feeds to have a proper evaluation. Some of the problems were obviously related to different buffer capacities in the incubated materials.

This was demonstrated by the results of Mougham et al. (1989) investigating 20 meat and bone meal samples. The authors compared the results obtained from the 4-enzyme method with in vivo true ileal protein digestibility obtained from rat trials. A significant relationship was found between the pH-drop and in vivo digestibility. However, meat and bone meal samples highly digestible in vivo caused a lower drop in initial pH, which is the opposite to what is expected and reported by other workers. However, a negative correlation between pH-drop and ash content in the samples suggested a strong buffering capacity of meat and bone meal due to its high mineral content (mean ash = 27.5%).

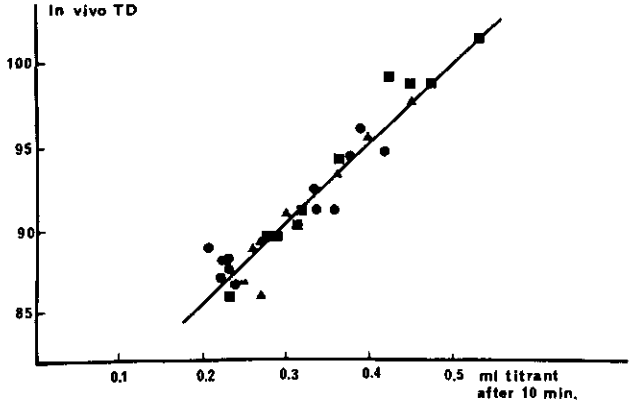

Fig. 1. Relationship between the amount of titrant (ml) added during 10 min and in vivo digestibility. Animal proteins ( ); plant proteins ( ); combinations ( ). From Pedersen & Eggum (1983).

Pedersen & Eggum (1983) performed the enzymatic digestion at a constant pH in a pH-stat. The uptake of titrant during the digestion (10 min. at pH 8.0) was correlated with true protein digestibilities determined in rats (Fig. 1). This technique improved the prediction of protein digestibility, and one could use only one regression equation for all samples. In general a good agreement was obtained with faecal true digestibility of plant proteins as well as with proteins of animal origin, and in samples having high buffer capacities. However, a predigestion step with pepsin was suggested for samples containing protease inhibitors. This study demonstrated that protein digestibility in a variety of materials could be estimated accurately when using the pH-stat procedure. However, certain physical characteristics of some proteins might prevent an accurate estimation of protein digestibility by this in vitro procedure.

Results obtained in a recent comparative study of in vitro measurements of 19 food products by the pH-stat method (Eggum et al., 1989) and dialysis-cell method (Savoie et al., 1989), respectively, were compared with with in vivo protein digestibility measurements using the rat balance method of Eggum (1973). The in vivo measurements were performed in two different laboratories (Eggum et al., 1989; Sarwar et al., 1989). In general, excellent agreements were obtained between results from all four measurements (Table 2).

Table 2. Values for protein (nitrogen) digestibility (%) of selected digestible products (Sarwar et al., 1989)

	In vitro digestibility			In vivo digestibility	
Products	Eggum et al. (1989)	Savoie et al. (1989)		Eggum et al.(1989)	Sarwar et al.(1989)
Casein	96	100[a]	94[b]	97	99
Skim milk	95	100	94	93	95
Skim milk (heated)	92	98	93	90	90
Tuna	92	99	98	93	97
Beef salami	95	114	102	96	99
Sausage	93	64	-	94	94
Chicken franks	95	107	94	96	99
Peanut butter	92	110	100	92	98
Soy protein	94	96	92	92	98
Pea protein	93	99	94	93	92
Pinto bean	90	80	83	73	79
Kidney bean	-	92	90	-	81
Lentil	-	86	86	-	84
Chick pea	100	87	82	88	89
Rolled oat	93	73	-	94	91
Wheat cereal	88	73	-	91	91
Rice-wheat-gluten	90	71	-	93	95
Macaroni-cheese	94	93	90	95	94
Beef stew	86	85	86	86	89

a) Relative digestibility (casein = 100).

b) Values obtained from regression equation $y = 39.42 + 1.22x$; $r = 0.78$, where x = in vitro digestibility as measured by digestion cell technique.

C. Undigested matter after incubation in a closed system

In vitro methods comprising enzyme incubations in a closed system followed by measurements of nutrients in the undigested (unsolubilized) residue - or alternatively in the filtrate or supernatant after centrifugation, include a large number of variants. These can be divided in single enzymatic systems and multi-enzyme systems performed in one, two, or three incubation steps.

Single enzymatic systems have been developed with pepsin (Sheffner et al., 1956) papain (Buchanan et al., 1969), trypsin (Maga et al., 1973), pronase (Taverner and Farrell, 1981), and rennin (Bhatty, 1982). Also two-enzyme systems have been reported, i.e. pepsin and trypsin (Saunders et al., 1973), pepsin and pronase (Dierick et al., 1985). However, multienzyme systems simulate in vivo conditions more closely, and as the degradation of most dietary components is influenced by the degradation of the other components, multienzyme systems are believed to give the best results.

During the last decade many approaches with different enzyme combinations and incubation conditions have been reported. These methods can be divided into five subgroups according to enzyme combinations (Table 3).

Table 3. Multi-enzyme systems used for in vitro incubations

1-step systems:
Intestinal fluids
2-step systems:
Pepsin - jejunal fluid
Pepsin - pancreatin
3-step systems:
Pepsin - pancreatin - rumen fluid
Pepsin - pancreatin - fibre-degrading enzymes

(a) Intestinal fluids

In vitro incubations with inocula from intestinal fluids were described by Goering & Soest (1970), Ehle et al. (1982), and Holzgraefe et al. (1985). Löwgren et al. (1989) described an in vitro system with 3 different inocula from a) duodenal fluid, b) ileal fluid, or c) faeces extract. Incubations were performed in a physiological buffer (pH = 6.9) at 38°C for 24 hours in procedure a) or 48 hours in procedure b) and c). The digestibilities (in vitro disappearences) of dry matter, energy, ash, crude protein, starch, dietary fibre, NSP and monosaccharides in 5 diets: basal, wheat bran, sugarbeet pulp, pea (early harvested), and pea (late harvested) were compared with in vivo values of ileal and faecal apparent digestibility (Graham et al., 1989). The authors concluded that this technique could be used to predict the availability of starch and crude protein for digestion in the small intestine, as well as the degradability of dietary fibre, and thus for comparing the nutritive value of pig feeds.

(b) Pepsin-jejunal fluid

Furuya et al. (1979) developed an in vitro method involving an 4-hour initial pepsin digestion followed by a digestion during 4 hours with pig intestinal fluid. A high agreement with in vivo faecal apparent digestibility of dry matter and crude protein was found for 7 diets. As this system attempts to simulate gastric and small intestinal digestion in pigs or other monogastric animals, Sakamoto et al. (1980) used the same procedure to estimate in vivo digestibility of dry matter and crude protein in diets for poultry.

However, other studies of this procedure by Clunies and Leeson (1984) gave unpredictable and variable results. These authors examined the method for routine analysis in quality control of feeds and feed ingredients for poultry, and concluded that this method would appear to have limited application for predicting overall digestibility in pig diets due to the fermentation in the large intestine.

(c) Pepsin-pancreatin

Incubations of samples with pepsin followed by pancreatin were used by Mauron et al. (1955) using a dialysis bag for the pancreatin incubation as described above. This combination was also used by Akeson & Stahmann (1964) and Büchmann (1979) using incubations in a closed system. In the method of Büchmann (1979) samples were incubated with pepsin dissolved in HCl (pH = 2.0) at 37°C for 6 hours. Then a buffered solution of pancreatin was added to obtain a resulting pH of 7.6 and than the incubation was continued for 18 hours. Dissolved proteins were precipitated with trichloroacetic acid, and after centrifugation the supernatant was evaporated to dryness at 80°C before determination of of undigested nitrogen. Good agreement between in vitro digestibility of protein and true digestibility values determined on rats was found for 30 barley samples while unsatisfactory results were obtained with other cereals.

Dierick et al. (1985) compared results obtained with three different two steps in vitro methods, all including pepsin in the first step and with jejunal fluid, pancreatin, or pronase in the second step. The in vitro results of protein digestibility in 30 feed ingredients and compound feeds were correlated to in vivo results of apparent ileal and faecal digestibility, and it was concluded that jejunal fluid can be replaced by an appropriate pancreatin solution without reducing accuracy. However, probably due to the fact that the in vivo data of feed ingredients were from literature values and thus not own data from identical samples, the relationship between in vivo and in vitro values were much lower than reported by Furuya et al. (1979). Furthermore, in vitro values were better correlated with faecal values than with ileal values which is probably also due to a great and varying amount of endogenous nitrogen at ileal level.

Babinszky et al. (1990) removed fat by pre-extracting samples with petroleum ether before incubations with pepsin and pancreatin, and compared this pre-extraction with an alternative addition of lipase and bile salt to the pancreatin. The authors found an improved correlation to the content of faecal digestible crude protein when using pre-extraction.

Boisen (1991a) improved the technique by using a standardized filtration equipment for measuring dietary fibre as described by Asp et al. (1983). Furthermore, chloramphenicol was added to the incubation

media to prevent bacterial growth, and finally soluble peptides and proteins were precipitated with sulphosalicylic acid before the removal of digested material by filtration. The obtained in vitro results of protein digestibility in a variety of feedstuffs were in general very close to the values of true protein digestibility determined in rats and pigs.

By this method in vitro digestibilities of individual amino acids were in general very close to each other as well as to nitrogen digestibility in eight common feedstuffs (Boisen & Fernàndez, 1991). When comparing to the apparent ileal digestibilities obtained at terminal ileum in fistulated pigs, the endogenous losses of N and amino acids were found to be related to undigested dry matter. Furthermore, dry matter digestibility when using this method were in good agreement with apparent ileal digestibilities of dry matter as well as of energy. Thus, the apparent ileal digestibilities of amino acids may be estimated from the in vitro measurements of true digestibility of nitrogen and in vitro undigested dry matter.

(d) Pepsin-pancreatin-rumen fluid

To have a complete feed evaluation system the potential energy utilization from fibre degraded by microbial enzymes must also be included in the in vitro system. As the bacterial population in the hindgut of pigs is similar to that in the rumen (Fonty & Gouet, 1989) the method of Tilley & Terry (1963) with bacteria isolated from a cow rumen was used by Vervaeke et al. (1989) for studying the hindgut fermentation after preincubating the material with pepsin and pancreatin. Good agreements with results from in vivo measurements were obtained.

(e) Pepsin-pancreatin-fibre degrading enzymes

Metz & Meer (1985) reported the results from initial studies on in vitro digestibility of organic matter in 20 feedstuffs and 20 mixed diets after incubations with pepsin (at pH = 1), pancreatin (supplied with lipase, bile salt, and the microbial amylase, Termamyl and cellulase, respectively. The method was concluded to be promishing for estimating faecal apparent digestibility of organic matter, but not yet accurate enough. A similar method was used by Meer & Perez (1990) in a study comprising 89 diets from 4 European countries. However, pepsin incubation was performed at pH 1.5 and no additions to pancreatin was made. Results from 4 laboratories were included after calibration of the method with samples of known in vivo digestibility. In this study organic matter digestibility was estimated with high accuracy and it was concluded that the method is an alternative to the prediction of in vivo digestibility from the chemical composition and digestibility coefficients obtained in trials with growing pigs. The addition of the natural logarithme of the crude fibre content as a variable in multiple regression analysis seemed to improve the accuracy of prediction.

Boisen & Fernàndez (1991) found a close relationship between results of dry matter digestibility obtained after incubation with pepsin pancreatin and rumen fluid according to Vervaeke et al. (1989) and after replacing rumen fluid (incubated for 48 hours) with Viscozyme (only incubated for 18 hours). Viscozyme is a multienzyme complex containing a wide range of carbohydrases including cellulase, hemi-cellulase, arabinase, xylanase, β-glucanase and pectinase. The degradation from this enzyme coctail corresponds to the potentially fermentable fibre.

Results from both methods were in good agreement with apparent faecal digestibility of dry matter and energy as well.

Based on the results obtained with pepsin and pancreatin and with pepsin, pancreatin and Viscozyme, respectively, Boisen (1991b) suggested an in vitro system of two parallel incubations for simulating the digestion in stomach and small intestine and the digestion in the hindgut, respectively (Fig. 2).

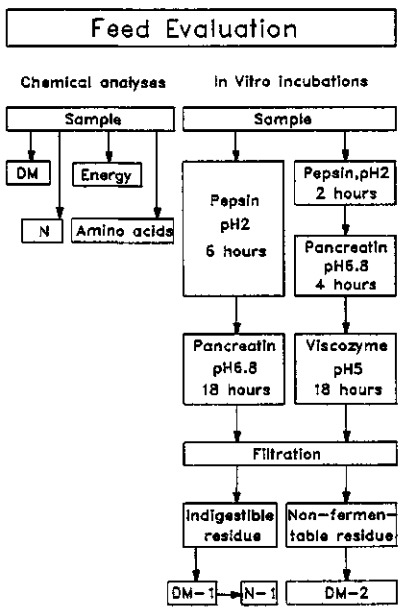

Fig. 2. A suggested system for feed evaluation based on chemical analyses and flow diagram of in vitro incubations for predicting ileal digestibility of dry matter (DM-1) and protein (N-1) and faecal digestibility of dry matter (DM-2). From Boisen (1991b).

Discussion

The requirements to a practical in vitro method for general and routinous use is that it shall be simple, quick, reproducible and give reliable results. Furthermore, the enzymes or enzyme mixtures used should be well-characterized and commercially available and finally, it would be desirable if the method was able to predict both protein and energy value of the feed.

Methods involving dialysis seem to be too complicated for routinous use and is more suited for studying the kinetics in proteolysis and amino acid release. The pH-drop method is influenced by the buffer capacity in the reaction mixture, which may result in misleading measurements. The pH-stat method seems to be useful for predicting protein digestibility in most feedstuffs which have a relatively high

digestibility. However, this method is meant only for protein digestibility. According to the many variants of closed incubation systems some general conclusions may be made:

a) multi-enzyme systems give a better simulation of in vivo conditions than single enzyme systems, and are therefore more reliable,

b) use of enzyme mixtures obtained from pancreas or intestinal fluid makes it possible to simulate the in vivo digestion of several nutrients,

c) compared to intestinal fluids pancreatin is
   - easy to obtain (commercially available)
   - constant and well-defined in composition (which increase reproducibility within the laboratory and especially between laboratories)
   - free from microbial enzymes.

Although intestinal fluid may simulate digestion in the small intestine more closely than pure pancreatin, it will be an advantage to measure digestion with the animals' own enzymes and microbial enzymes separately. This suggestion is based on the lower utilization of energy from microbial fermentation compared to that from carbohydrates digested in the small intestine. The utilization depends on dietary fibre composition with a variation from 50-80% (Dierick et al., 1989).

The fermentability of fibre can be measured by incubation with rumen bacteria. However, the use of a mixture of fibre-degrading enzymes has several advantages, as they are commercially available and more constant and well-defined in composition. Furthermore, the incubation time can be reduced considerably.

In general fibre and antinutritional factors (ANF) like trypsin inhibitors, tannins, lectines, glucosinolates, and alkaloids depress the nutrient digestibility in vivo, but have much less or no effect on in vitro values. This is due to a quite different action of these substances on in vivo and in vitro conditions. However, the results of Poel (1990) clearly demonstrate that these problems are not overcome by the mobile nylone bag technique, although this technique may be considered as an intermediate between in vivo and in vitro methods. Therefore, the results from this method seem generally not to be more correct than results obtained from well-defined and well-controlled in vitro conditions.

In vitro results generally correspond to the maximal digestibility values when not depressed by ANF. To compensate for this, additional specific analyses are required. On the other hand, it is also a task for the feed producer to make sure that the level of these substances is so low that they only have minor or no influence on the in vivo digestibility.

The control of commercial feed mixtures based on in vitro digestibility of protein and energy should therefore also include specific analyses for ANF.

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A DISCONTINUOUS IN-VITRO TECHNIQUE FOR MEASURING HINDGUT FERMENTATION IN PIGS

F. Ahrens, Jutta Schön, M. Schmitz

IS Forschungsgesellschaft für experimentelle Tierphysiologie und Tierernährung, Wahlstedt, Germany

Abstract

The in-vitro technique of Menke for ruminal fermentation was modified and adapted to the hindgut fermentation of pigs. Therefore in smaller calibrated syringes 5 ml fresh caecal fluid, 5 ml buffer and centrifuged and freeze dried caecal content as the substrate were incubated at 39° C. As donors for the caecal content 6 adult miniature pigs with a permanent caecal cannula were used. Per treatment 3 syringes were used, and the set was repeated on a second day. At the end of the incubation pH, ammonia concentration, single and total VFA were determined in each syringe. Testing increasing amounts of substrate (100, 200 or 300 mg per syringe) showed good accordance of 200 mg with in-vivo data. So, for further experiments this amount was chosen. Variating the incubation time from 6 to 12 to 24 h showed as under in-vivo conditions (at different time after feeding) decreasing pH values, increased level of total VFA and a change in the fermentation pattern towards higher propionic acid production. But, in contrast to in-vivo data the ammonia concentrations increased unter the in-vitro conditions. On the other hand, like in-vivo decreased ammonia concentrations were observed also in-vitro, when increasing the fermentation by supplementing pectin or lactitol. When supplementing these two substances as examples for fermentable carbohydrates in increasing amounts (5 to 20 %) to the 200 mg substrate, they were fermented in the same way as under in-vivo conditions. First results from a direct comparison with in-vivo hindgut fermentation in minipigs showed very similar data after 24 h in-vitro fermentation and measuring in the caecum 8 h after the morning meal.

In conclusion the described in-vitro technique seems suitable for measuring changes in hindgut fermentation in pigs, caused by a more intensive fermentation. Further experiments have to be done to measure the effects of other substances, e. g. growth promoters, organic acids and probiotics on hindgut fermentation.

Introduction

Measuring hindgut fermentation in pigs becomes more and more of interest, because the bacterial transformation of carbohydrates, proteins and other substrates in this part of the digestive tract has an influence on the digestibility, especially of complex carbohydrates and the growth and health of pigs. In-vivo trials to measure hindgut fermentation are costly and time consuming. But in contrast to ruminants, where in-vitro techniques for measuring rumen fermentation have been widely used, there are only a few attempts to simulate hindgut fermentation in pigs. Further, most of these studies are carried out to measure digestion of dry matter (Holzgraefe et al.,1985, Löwgren et al.,1989), or fibre (Ehle et al.,1982) or the influence of energy metabolism (Vervaeke et al.,1979).

In our experiments we focus our interest more on simulating the effects of intensified hindgut fermentation on parameters as pH, ammonia concentration and pattern of volatile fatty acid production which directly or indirectly influence the health of the host.

Materials and Methods

Technique: The in-vitro technique of Menke et al.(1979) for measuring ruminal fermentation was used with some modifications. The incubations were carried out in smaller calibrated syringes (75 ml volume, 50 ml calibrated). The ratio of inoculum to buffer was increased from 0.5 to 1 in the original test to 1 : 1 for the present purpose.

Animals: 6 male adult Göttingen Miniature pigs with a permanent T-shaped cannula in the caecum were used as donors for the substrate and the inoculum. They were maintained on a grain/soyabean meal diet (18 % crude protein, 3 % crude fibre) which was fed in two equal amounts at 7 a.m. and 5 p.m.

Substrate: As substrate for the in-vitro test centrifuged (the supernatant was refused) and freeze diet caecal content from all 6 pigs was used. Chyme was collected in the morning between 8 and 10 a.m. The dried and milled (0.5 mm screen) content of 3 to 5 days was pooled to get enough material of the same composition for a set of in-vitro experiments.

Inoculum: Fresh centrifuged (5 min. 100 x g) caecal fluid of all 6 pigs collected in the morning. 5 ml caecal fluid per syringe were used.

Incubations: The incubations were carried out at 39° C in a drying oven. The syringes were placed in a rotating incubation apparatus. Per treatment 3 syringes were incubated, and the set was repeated on a second day. At the end of the incubation fermentation was stopped by putting the syringes in ice cold water. In the content from each syringe pH, concentration of ammonia, of acetic, propionic, n-butyric and iso-acids (butyric and valeric) as well as total concentration of these volatile fatty acids (VFA) were determined (ammonia: colorimeter, VFA: gas chromatography).

Experimental design: The following treatments were carried out:
- influence of the amount of substrate: 100, 200 or 300 mg/syringe;
- variation of incubation time: 6, 12 or 24 h;
- influence of supplementation of fermentable carbohydrates: 5, 10 or 20 % of pectin or lactitol were mixed with the substrate. Up to 20 % were supplemented, because this correspondences approximately to 5 % of pectin in the diet (80 % digestibility of the nutrients at the end of small intestines leads to an increase of the pectin concentration in the chyme, when entering the hindgut to 20 %, because the pectin is not digested in the small intestine);
- comparison with in-vivo trials: for this purpose the in-vitro results obtained using the pectin were compared with data obtained in the caecum of 6 minipigs which got the same diet mentioned above, but supplemented with 5 % of pectin. The in-vivo experiment was carried out as an cross-over design with 2 periods of 3 weeks each. After 11 days for adaptation to the diets on 5 different days caecal content was sampled before the morning meal (0 h), 4 and 8 h later for measuring the same parameters as in the in-vitro experiments.

Results and Discussion

Influence of the amount of substrate: The results in table 1 show that the addition of 100 mg of dry caecal content resulted in comparison with an incubation without substrate (blanc) in a decreased pH, increased ammonia concentration and total VFA concentration. The fermentation pattern was shifted towards propionic acid production as can be taken from the last column in table 1. Increasing the amount of substrate to 200 and 300 mg resulted in a further decrease in pH and C_2 to C_3 ratio. The concentration of ammonia and total VFA were increased.

Table 1. Influence of amount of substrate on in-vitro caecal fermentation (24 h incubation, 2 days; n = 6 syringes total, x +/- s)

amount substrate[1] (mg/syringe)	pH	ammonia (mmol/l)	total VFA (mmol/l)	C_2 : C_3 ($\bar{x}$: 1)
blanc[2]	6.82	5.27	34.8	3.67
	0.01	2.00	4.6	0.12
100	6.64	11.77	64.9	3.28
	0.03	0.84	3.6	0.10
200	6.49	17.80	91.4	3.18
	0.02	1.48	5.6	0.10
300	6.31	27.10	120.2	3.06
	0.05	1.05	3.8	0.06

1 substrate: centrifuged, freeze dried caecal content.
2 blanc: incubation of caecal fluid and buffer only.

Because the values obtained after incubation of 200 mg substrate fitted well with in-vivo data (Ahrens and Kaufmann, 1985) this amount was chosen in the following experiments.

Variation of incubation time: Incubation of 200 mg of dry caecal content with 5 ml fresh caecal fluid and 5 ml buffer over periods of 6, 12 or 24 hours resulted in decreased pH values (0.1 to 0.3 units) with increased incubation time. The level of ammonia (+ 2 to + 7 mmol/l), propionic acid and total VFA (+ 10 to + 30 mmol/l) was increased, the level of acetic acid and C_2 to C_3 ratio decreased (-0.3 to -0.6 : 1). These results are in accordance with in-vivo data. The only exception are the results for the concentration of ammonia which is lower under in-vivo conditions because of the rapid absorption from the hindgut and the incorporation into bacterial protein (Ahrens and Kaufmann, 1985).

Influence of fermentable carbohydrates: The results in table 2 show, that both, pectin and lactitol, caused a dose dependent decrease of ammonia level and pH in comparison to the control. With increasing dose of both carbohydrates the total VFA concentration as well as the levels of acetic and propionic acid increased also.

Table 2. Influence of pectin and lactitol on in-vitro hindgut fermentation (6 h incubation; mean of 6 syringes)

Treatment	ph	ammonia (mmol/l)	total VFA (mmol/l)	acetic (mmol/l)	propionic (mmol/l)
control[1]	6.80	9.7	56.0	36.4	10.8
+ 5 % Lactitol	6.73	8.0	63.8	42.3	12.3
+ 10 % Lactitol	6.60	6.1	74.4	50.5	14.4
+ 20 % Lactitol	6.32	2.5	92.3	63.3	18.6
+ 5 % Pectin	6.75	8.8	64.3	43.3	12.1
+ 10 % Pectin	6.66	6.9	70.8	49.5	12.5
+ 20 % Pectin	6.45	4.3	80.7	58.5	13.4

[1] control = 200 mg dry caecal content

For all parameters the effect was more pronounced with the lactitol. Further, the rise in propionic acid was higher with the lactitol (from 12.3 to 18.3 mmol/l with 5 and 20 % addition of lactitol, respectively) than with pectin (from 12.1 only to 13.4 mmol/l). This demonstrates a different fermentation pattern of lactitol and pectin and is again in accordance with the literature (Kass et al., 1980; Kim et al., 1978). The decrease of the concentration of ammonia after adding fermentable carbohydrates fits very well with in-vivo data (Ahrens and Kaufmann, 1985).

Comparison with in-vivo trial: When comparing the in-vitro data with in-vivo data in the experiments where the fermentable carbohydrate pectin was used, first results show very similar data between a 24 h in-vitro incubation with 20 % of pectin and a sampling of caecal content 8 hours after feeding a diet with 5 % pectin (see table 3).

Table 3. Comparison of in-vitro and in-vivo data of hindgut fermentation (Differences from control, obtained after 24 hours in-vitro incubation or in-vivo 8 hours after feeding)

	ph	ammonia (mmol/l)	total VFA (mmol/l)	$C_2 : C_3$ (x : 1)
Pectin				
in-vitro 20 %	- 0.3	- 3.5	+ 27	+ 0.5
in-vivo 5 %	- 0.5	- 4.5	+ 29	+ 0.5

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IN VITRO DIGESTIBILITY OF ENERGY AND AMINO ACIDS IN PIG FEEDS

S. Boisen and J.A. Fernàndez

National Institute of Animal Science, Foulum, Denmark

Abstract

A two-step enzymatic in vitro method with pepsin and pancreatin incubations to estimate precaecal digestibility of dry matter and N, and a three-step enzymatic method with incubations of pepsin, pancreatin and a microbial fibre-degrading enzyme complex to estimate postileal digestibility of dry matter is described. In vitro digestibility of dry matter in eight common feedstuffs (barley, rye, wheat, oats, soybean meal, rapeseed meal, sunflower meal, and grass meal) was highly correlated with values of ileal and faecal digestibilities of dry matter as well as energy. In vitro digestibility of N corresponding to true digestibility of N, agreed generally with in vitro digestibility of the individual amino acids. From the differences between in vitro and in vivo values of digestible N and amino acids the endogenous losses of N and amino acids were calculated. The losses were linearly correlated to undigested dry matter in the two-step procedure. Based on these results a model for calculating net digestibility, corresponding to apparent ileal digestibility, of N and amino acids in feed mixtures is proposed.

Introduction

A precise feed evaluation require primarily knowledge of the contents of digestible (or better utilizable) energy and amino acids in the feeds. As the digestibilities vary considerably from one feedstuff to another and may also vary in different batches of the same feedstuff it could be most desirable to estimate the digestibility by an easy and reliable laboratory method.

Especially, during the last decade many approaches have been made to estimate the digestibility of nutrients by in vitro methods. However, most methods are used for the estimation of the digestibility of only one nutrient, mainly protein. Although the in vitro conditions in most approaches simulate the stomach and small intestine the results have commonly been compared with in vivo data of faecal digestibilities (Eggum & Boisen, 1991).

However, a significant microbial fermentation in the hindgut of influences 1) the utilization of energy, 2) the faecal contents of pigs' amino acids. Although most of the fermentation end products (VFA) contribute to the energy supply of the host animal, the utilization of this energy is much lower than that of the energy absorbed in the small intestine (Just et al., 1983). Amino acids are only absorbed in the small intestine (Just et al., 1981) and during the passage of the hindgut undigested amino acids may be either degraded or incorporated in microbial protein.

The determination of ileal digestibility of energy and amino acids combined with the faecal digestibility of energy is therefore assumed to give the best basis for a precise feed evaluation.

Thus, the objective of the present study was to investigate the possibility for the development of a reliable in vitro method which is

able to estimate the precaecal digestibility of energy and amino acids and also the postileal degradation (fermentation) of energy in feedstuffs as well as of feed mixtures with unknown composition.

Materials and Methods

Feeds

Samples of eight common feedstuffs (barley, rye, wheat, oats, soybean meal, rapeseed meal, sunflower meal, and grass meal) were analyzed in vitro. All samples were representative alliquots from experimental diets used in previous trials with ileum-fistulated pigs. The samples were stored in a deep-freezer until the in vitro assays were performed. All in vitro data are mean values of two measurements performed in two different days.

The in vivo digestibility trials were performed with pigs of about 50 kg as described by Just et al. (1985). Cereals were fed as the only feedstuff while the protein sources were fed together with a protein-free basal diet containing (g/kg): maize starch 660, potato starch 165, cellulose 75, animal fat 100. All in vivo data are mean values ± SD from 5 digestibility trials.

In vitro technique

A. Simulating precaecal digestion

The in vitro incubation conditions simulating the digestion processes in the stomach and small intestine with pepsin followed by pancreatin were a modification of the dietary fibre method of Asp et al. (1983). This method was modified to measure in vitro protein digestibility by changing enzyme concentrations, incubation time and pH in the incubation mixture.

Step 1. A series of 20 samples with about 1 g of finely ground material (ground to pass a screen with a pore size of 1 mm) were weighed to an accuracy of ± 0.1 mg in 100 ml conical flasks. In each serie a blank was included. A small magnetic rod and 25 ml of phosphate buffer (0.1 M, pH 6.0) was added to each flask and sample and buffer were mixed carefully by gentle magnetic stirring. The mixture was added 10 ml 0.2 M HCl and pH was adjusted to pH 2 with a 1 M HCl (or a 1 M NaOH-solution). The mixture was then added 1 ml of a freshly prepared pepsin solution, containing 10 mg pepsin (porcine, 2000 FIP-U/g, Merck no 7190). In order to prevent bacterial growth, especially during the second incubation step, 0.5 ml of a chloramphenicol solution (0.5 g/100 ml ethanol) was added. Then the flasks were closed with a rubber stopper and the samples incubated in a water bath at 39°C for 6 hours with repeatedly gentle magnetic stirring.

Step 2. The mixture was added 10 ml of a phosphate buffer (0.2 M, pH 6.8) + 5 ml of a 0.6 M NaOH-solution and then adjusted to pH 6.8 with a 1 M HCl or a 1 M NaOH-solution. The slurry was then carefully mixed with 1 ml of freshly prepared pancreatin solution containing 50 mg porcine pancreatin (porcine, grade IV, Sigma no P-1790). After closing with a rubber stopper, the flasks were placed in a water bath at 39°C for incubation overnight (18 hours).

To all samples were added 5 ml of 20% sulphosalicylic acid. Solubilized, but not digested, proteins were precipitated during 30 minutes of incubation at room temperature. The undigested residues were then collected in a filtration unit for crude fibre determination (Fibertec System M, Tecator, Sweden) by using dried and preweighed glasfilter crucibles (d: 3 cm: pore size: 40-90 µ) containing about 0.5 g Celite as filteraid. All material was transferred with 1% sulphosalicylic acid to the crucible, and the samples were dried at 80°C overnight. Then Celite and undigested material was wrapped into a piece of nitrogen-free paper and undigested nitrogen was measured by the Kjeldahl method in an automatic Kjelfoss apparatus (Foss Electric, Denmark).

In vitro digestibilities of dry matter (DM-1) and protein, respectively, were calculated from DM and N in sample and undigested residue, respectively, after correction for DM and N in the blank.

B. Simulating digestion in the whole gastro-intestinal tract

The in vitro incubation conditions simulating the whole gastro-intestinal tract was performed by adding an extra incubation step simulating the degradation of undigested carbohydrates, mainly fibre, in the hindgut.

Step 1 and 2. The incubations in step 1 and step 2 with pepsin and pancreatin, respectively, were performed as described in procedure A, except that the incubation times were shortened down to 2 and 4 hours, respectively. This reduction had no influence on the final dry matter digestibility and allowed the two in vitro procedures to be performed parallely. Furthermore, no sulphosalicylic acid was used for protein precipitation after step 2, and undigested materials were collected in the filtration unit by transferring to crucibles (see above) with water.

Step 3. The crucibles were closed in the bottom with a rubber stopper, and the undigested material was incubated with 20 ml of a freshly prepared solution of fibre degrading enzymes containing 0.4 g Viscozyme (120L, Novo, Bagsværd, Denmark) in 0.1 M Acetate buffer, pH 5. The enzyme solution was thoroughly mixed with the undigested material (and Celite) and then the crucibles were placed in an oven at 40°C for incubation overnight (18 hours).

After incubation the rubber stopper was removed and crucibles were placed in the filtration unit. After filtration and washing with water, the residue was dried at 80°C overnight, and undigested dry matter (DM-2) was measured as described in procedure A.

Results and Discussion

The in vitro digestibilities of dry matter (DM-1 and DM-2) by the two procedures A and B were, except for those of rye, highly correlated to ileal and faecal digestibilities of dry matter ($r = 0.97$; Table 1 and 2). The correlations of in vitro digestibilities of dry matter with ileal and faecal digestibilities of energy were of similar magnitude (Table 2).

According to Lekule et al. (1990) digestibility values of dry matter and energy in a wide range of feedstuffs are highly correlated to each other. Thus, in vitro values of dry matter digestibility may generally be used for predicting values of energy digestibility.

Table 1. In vitro digestibility of dry matter (DM-1 and DM-2) compared with in vivo digestibility of dry matter (DM-i and DM-f) and energy (E-i and E-f) in 8 common feedstuffs in pig feeds.

	In vitro	In vivo (ileum)		In vitro	In vivo (faeces)	
	DM-1[a]	DM-i	E-i	DM-2[b]	DM-f	E-f
Barley	70	71±2	72±3	82	80±1	81±1
Rye	80	68±2	70±3	89	82±1	82±1
Wheat	72	74±5	73±4	89	84±2	85±2
Oats	68	70±10	68±12	68	66±3	67±3
Soybean meal[c]	69	65±2	69±1	87	86±3	88±3
Rapeseed meal[c]	62	59±5	63±5	79	75±2	76±3
Sunflower meal[c]	58	54±4	60±3	74	71±1	72±1
Grass meal[c]	57	50±7	54±6	73	67±2	66±2

[a] incubation with pepsin and pancreatin, respectively
[b] incubation with pepsin, pancreatin and Viscozyme, respectively
[c] in N-free basal diet (see Materials and methods)

Table 2. Relation between in vivo dry matter and energy digestibility and in vitro dry matter digestibility. Data from Table 1 (rye excluded).

n	Equation[a]	r^2	RSD
7	DM-i = -32.5 + 1.47 DM-1	0.95	1.5
7	E-i = -6.5 + 1.11 DM-1	0.95	1.5
7	DM-f = -4.6 + 1.02 DM-2	0.95	1.9
7	E-f = -8.1 + 1.07 DM-2	0.92	2.5

a: abbreviations: see Table 1

The in vitro digestibilities of DM-2 seem in general to slightly overestimate the faecal energy digestibility. This may partly be explained by a more effective degradation of fibre than normally occuring in 50 kg pigs. Probably the in vitro degradation more closely corresponds to the fermentation occuring in sows, and reflects the potential degradation rather than the actual fermentation in growing pigs. On the other hand, diets for growing pigs usually contain relatively small amounts of fibre, limiting this source of error.

The in vitro digestibility of N was in all feedstuffs considerably higher than in vivo digestibilities determined in ileal digesta as well as faeces (Table 3). This is due to the contribution of endogenous N in the in vivo determinations resulting in apparent digestibilities, whereas the in vitro values correspond to true digestibilities. Thus, the endogenous losses of N might be calculated from the differences between in vivo and in vitro measurements (Table 3). Such calculations give considerable variations in the endogenous losses. However, these losses were closely related to the content of fibre in the feeds, and also to the amount of undigested dry matter in ileal digesta (Fig. 1).

Based on these results, the endogenous loss of N (g/kg DM intake) can be calculated from in vitro undigested dry matter (UDM, g/g intake) by a linear equation: $y = k * UDM$. Thus, in vitro net digestible N (DN_{NET}), corresponding to apparent ileal digestible N can be calculated from in vitro values of true digestibility of N (ND_{IV}, g/g intake) and UDM by the equation:

$$DN_{NET} = N_{intake} * ND_{IV} - k * UDM$$

Table 3. In vitro digestibility of nitrogen compared with in vivo apparent digestibility values of nitrogen in 8 common feedstuffs, and calculated values of endogenous losses of nitrogen in the small intestine and in the whole digestive tract, respectively.

	N in feed (g/kg DM)	Digestibility (%) in vitro	Digestibility (%) in vivo[a] ileal	Digestibility (%) in vivo[a] faecal	Endogenous loss (g N/kg DM intake) ileal	Endogenous loss (g N/kg DM intake) faecal
Barley	19.0	85	70±5	77±2	2.9±0.9	1.5±0.4
Rye	18.2	87	65±4	73±3	4.0±0.7	2.6±0.6
Wheat	23.2	91	74±8	85±2	3.5±1.9	1.4±0.5
Oats	18.2	89	61±5	76±3	5.0±0.9	2.4±0.6
Soybean meal[b]	29.1	92	78±3	80±2	4.1±0.9	3.5±0.6
Rapeseed meal[b]	31.2	83	69±2	72±3	4.4±0.6	3.4±0.9
Sunflower meal[b]	31.4	90	73±7	77±2	5.3±2.2	4.1±0.6
Grass meal[b]	14.2	74	35±18	37±1	5.5±2.5	5.3±0.1

[a]see Just, Fernandez and Jørgensen (1985)
[b]in N-free basal diet (see Materials and methods)

The in vitro digestibilities of individual amino acids were generally very close to the in vitro digestibility of N in the feedstuff. The digestibility of the most often limiting amino acids (lysine, methionine + cystine, threonine) is primarily of interest. Values of methionine tended to be slightly higher than values of N in most feedstuffs, while values of cystine were markedly lower in soyabean meal and sunflower meal. However, values of lysine and threonine differed only little or not at all from the N digestibility.

Due to the endogenous losses of amino acids, the in vitro digestibility values were considerably higher than apparent ileal

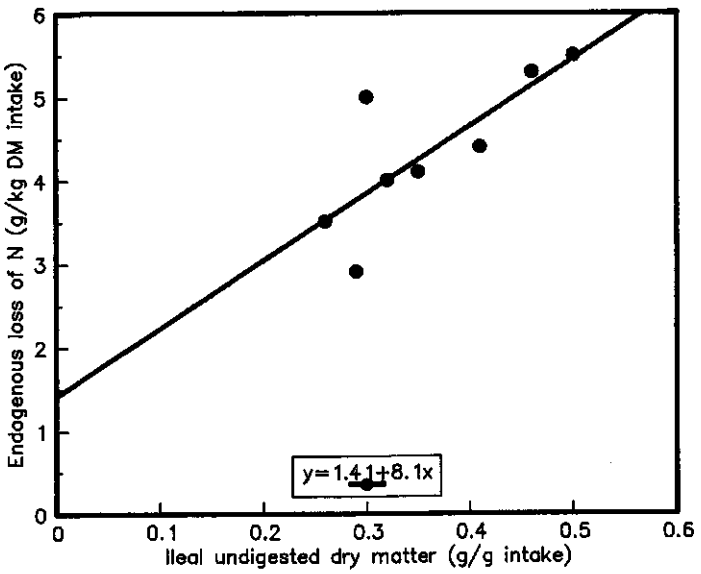

Fig. 1. Relationship between calculated endogenous loss of N and ileal undigested dry matter in pigs fed eight common feedstuffs.

digestibility values (partly published in Just et al., 1985). The calculation of these losses as differences between in vitro and in vivo digestibilities demonstrated that these losses - like the loss of endogenous N - were related to undigested dry matter. However, the composition of essential amino acids in the calculated endogenous protein was relatively constant and the mean values were very close to the mean values from literature values of in vivo determined protein losses (Table 4) as collected by Wünche et al. (1987).

Table 4. Amino acid composition (g/160 g N) of endogenous protein calculated from differences between in vitro and in vivo digestibilities compared with direct in vivo measurements.

	lys	met	cys	thr	ile	leu	his	phe	tyr	val
In vitro/in vivo	28	8	16	40	23	35	10	28	22	33
In vivo[1)]	27	7	15	37	18	36	13	23	18	29

1) calculated from Wünche et al. (1987).

Thus, assuming identical digestibilities of N and amino acids and a constant amino acid composition in the endogenous protein, in vitro net digestible amino acids can be calculated in a similar way than in vitro net digestible N by using conversion factors from N to individual amino acids in the endogenous protein.

The reliability of predicting ileal and faecal digestibility of energy from in vitro digestibility of dry matter and the adequacy of the model for predicting apparent ileal digestibility of N and amino acids is currently being investigated in a wide range of feed mixtures.

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IN VITRO ESTIMATION OF READILY AND LESS-READILY AVAILABLE NUTRIENTS

W. Lowgren[1] & H. Graham[2]

[1]Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden
[2]Finnfeeds International Ltd., Forum House, Redhill, Surrey RH1 6YS, England

Abstract

An in vitro method for the study of digestion of feeds in the pig gastro-intestinal tract has been developed. This method is based on the incubation of feed samples with buffered duodenal, ileal and faecal inocula, and it would appear that there is little difference in the pattern of degradation between these inocula. In vitro disappearance was little affected by donor age or diet, and the variation in inocula activity within pig was generally greater than that observed between pigs. Sample particle size affected the rate but not the final extent of in vitro disappearance. This method can be used to study the rate and extent of degradation of different feed components. Short incubation times of about 12 h can be used to compare or predict in vivo ileal apparent digestibilities of dry matter, starch and protein, while 48 h incubations can predict the in vivo faecal apparent digestibility of dry matter and dietary fibre. The DE and ME contents of diets can also be predicted by this method.

Introduction

The determination of digestibility by conventional methods requires large quantities of feed, a number of animals and considerable expenditure on equipment and manpower. Further, there is a considerable body of public opinion which is not in favour of animal experimentation, regardless of whether these trials are stressful to the animal. Thus in recent years there has been an increasing interest in developing rapid laboratory methods for the study of digestion in the pig and the evaluation of pig feeds. Basically such methods may be divided into three groups:

1. Chemical methods
2. In sacco methods
3. In vitro methods

While chemical methods may be used to indicate the nutritive value of feeds, they will not give any information on the kinetics of digestion or predict the effects of processing on digestibility (Graham & Lowgren, 1991). In sacco methods, where a sample of feed contained in a small bag is introduced into the intestine of the animal and collected in the faeces, may also give some idea of the relative digestibility of feeds (Graham et al., 1985). However, such methods require cannulated animals and again give no information on digestion rates. In vitro methods have few of the disadvantages of these methods, and thus a number have gained prominence in recent years.

Generally in vitro methods are based on the degradation of feed samples, either by enzyme cocktails or by intestinal inocula, with recovery of the undegraded residue by filtration or centrifugation. Obviously such methods are limited in that all soluble material and small particles must be assumed to be degradable. Also the influence of anti-nutrients will often be lost as will the effect of feed components on animal factors such as endogenous production and digesta transit time, which will affect apparent digestibility in vivo. The use of intestinal inocula rather than enzymes should give a more realistic reflection of the processes within the gastro-intestinal tract where some microbial degradation takes place even in the fore-gut (Graham et al., 1986). This degradation may play an important role in releasing other nutrients for enzymatic digestion in the small intestine.

Materials and Methods

The in vitro method employed was based on that of Furuya et al. (1979). Generally fresh duodenal digesta, ileal digesta and faeces were collected from donors, filtered through cheese cloth and diluted to 20 % in physiological buffer (Lowgren et al., 1989). Fifty ml of these buffered inocula were then incubated with 0.5 g feed sample at 38°C under anaerobic conditions for periods from 1 to 96 h. The undegraded residues were recovered by filtration, extensively washed with water, dried and weighed.

Degradation in vitro

Initial studies established that this in vitro method was applicable to pig feeds, and that a short incubation time could be used to predict the readily-available nutrients and longer incubations the less readily-available components (Lowgren et al., 1989). It was also apparent that degradation by duodenal, ileal or faecal inocula differed in rate but not pattern; ie, all inocula degraded individual feed components in the same order. However, faecal inocula tended to give a lower apparent disappearance of protein, possibly due to microbial contamination of the residue, and ileal and faecal inocula

tended to have a higher initial capacity to degrade fibre components. Feed particle size was also shown to influence the rate of disappearance in vitro but not the extent of disappearance at longer incubation times.

Subsequent studies employing 5 feeds that had been evaluated in vivo (Graham et al., 1989) indicated that a 12 h incubation with duodenal inocula could be used to compare the in vivo ileal apparent digestibilities of dry matter, starch and crude protein (Table 1). A 48 h incubation with ileal or faecal inocula could also be used to compare both the extent and pattern of degradation of fibre components. However, in vivo faecal apparent digestibility of crude protein was not related to in vitro protein disappearance, indicating the influence of endogenous losses on this component in vivo.

Table 1. Correlation coefficients (x100) between in vivo ileal and faecal apparent digestibilities (%) and in vitro disappearance with duodenal (DD, 12 h), ileal (ID, 48 h) and faecal (FD, 48 h) inocula for different components of 5 pig feeds.

In vivo	DD	ID	FD
Dry matter			
ileal	89		
faecal		84	81
Crude protein			
ileal	88		
faecal		<50	<50
Starch			
ileal	96		
faecal		<50	<50
Dietary fibre			
ileal	<50		
faecal		94	94

A study of 11 pig feeds indicated that there was a close indirect correlation between dietary starch content and the content of the other main components, which could be expected as starch is generally the predominant dietary component (Table 2). Starch content was also closely related to in vitro disappearance with ileal inocula. However the relationship with faecal in vitro disappearance was less strong, possibly due to difficulties in weighing the small amount of residue present after degradation and to the fact that the faecal inocula contains small particles which can be difficult to wash from this residue. This study also established that both analytical and in vitro data can be used to predict the DE and ME content of pig feeds (Graham & Lowgren, 1991).

Table 2. Correlation coefficients (x100) between in vitro disappearance (48 h) with ileal (ID) and faecal (FD) inocula and the composition of 11 pig feeds.

	Protein	Fat	Fibre	Ash	ID	FD
Starch	-88	-90	-86	-76	86	44
Crude protein		69	55	46	-57	-7
Crude fat			91	71	-87	-47
Dietary fibre				86	-98	-75
Ash					-91	-78
ID						79

An in vitro method has been developed which allows the study of the digestion of pig feeds. This method can be used to predict the ileal apparent digestibility of starch and crude protein and the faecal degradation of fibre, which should be essential components of any such method. Inclusion of appropriate standard feeds and the use of standardized inocula or enzymes will allow comparisons between laboratories and different runs. All such methods have disadvantages, with the influence of anti-nutritive factors and endogenous losses in vivo particularly difficult to account for. However, providing that these limitations are taken into consideration, such methods will prove useful in the study of the processes of digestion and the nutritive value of pig feeds.

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A COMPARISON OF IN VIVO AND IN VITRO METHODS FOR ESTIMATING DIGESTIBILITY IN SWINE

C. Bellaver,[1] R. A. Easter,[2] D. A. Cook[3] & A. Gonzalez[4]

Department of Animal Sciences, University of Illinois, Urbana, Illinois, USA & EMBRAPA Empresa Brasileria De Pesquisa Agropecuá, Brazil

Abstract

Three methods for predicting amino acid utilization: two in vivo procedures, swine ileal sampling and precision-fed cecectomized rooster excreta collection, were used to obtain digestibility values, and an in vitro enzyme incubation method was used to estimate potential protein hydrolysis. The feedstuffs used in each assay were: corn, wheat bran, two soybean meals, cottonseed meal, poultry by-product meal and two meat and bone meals. A common sample of each feedstuff was used for each of the experimental procedures. Lysine, threonine and tryptophan digestibilities were consistently lower when measured by swine ileal than by precision-fed rooster procedures. The correlations among methodologies were low when low-protein feedstuffs were included in the data set, but were improved when only high-protein feeds were included.
Keywords: amino acid utilization, amino acid digestibility, amino acid availability

Introduction

Published amino acid requirement estimates assume (1) that dietary amino acids are completely utilized or, (2) that utilization is less than complete and that adjustments will be made to allow for differences in utilization between that found in the diets used to determine the requirement and that in the ingredients being used in formulation. Accurate estimates of amino acid digestibility (or availability) are needed before these adjustments can be made.

There has been much attention given to the estimation of amino acid digestibility (or availability) by various methods. This effort is a result of the concept that digestible amino acid values can be used effectively in diet formulation. These procedures have been reviewed by Low (1982), Sauer and Ozimek (1985) and Sibbald (1987). The present experiments were undertaken to obtain estimates of the digestibility of certain essential amino acids using swine and rooster procedures and an "in vitro" enzymatic digestion method. The following ingredients were evaluated: corn, wheat bran (WHB), soybean meal (SBM), cottonseed meal (CSM), poultry by-product (PBP) and meat and bone meal (MBM). These values were used to establish correlations among the different methods and to test the hypothesis that diets formulated with different ingredients to the same concentrations of digestible amino acids would support equal swine growth performance.

1. Present address: C. Postal 21, 89700 Concórdia SC, Brazil.
2. Professor at the University of Illinois, Champaign-Urbana.
3. Graduate research assistant.
4. Present address: San Juan, Costa Rica.

Materials and Methods

Swine ileal digestibility

Nine crossbred barrows from three litters of Yorkshire x Hampshire parentage initially weighing 42.44 kg ± .46 were surgically fitted with individual T-cannulae. After 14 days of post-surgical recuperation (Zebrowska, 1973; and Lin et al., 1987), procedures to measure ileal digestibility were initiated. Pigs were housed in stainless steel metabolism cages during both the adaptation and collection periods in a temperature-controlled environment. Pigs were allowed a five day period of diet adaptation (Tanksley and Knabe 1984) followed by two days of chyme collection (Taverner et al., 1981a; Sauer et al., 1982; Partridge et al., 1987). They were fed at 12-hour intervals (7 a.m. and 7 p.m.) throughout the experiment and digesta was collected during the 12 hour period between the morning and evening meals on each of the two days (Tanksley and Knabe, 1984; Taverner et al., 1981a). Diets were formulated to provide similar levels of metabolizable energy, calcium and phosphorus (except for sources of animal proteins), vitamins, and minerals (NRC, 1979).

Percentage true amino acid digestibility (TAAD) was calculated according to Sauer and Ozimek (1985). A nitrogen free diet (NFD) was used to estimate the endogenous amino acid losses.

Precision-fed rooster assay

True amino acid digestibilities (TAAD) were measured for a number of ingredients using the procedure of Sibbald (1979) as modified by Parsons (1985b). Three mature Single Comb White Leghorn cockerels per treatment were allocated to individual cages with raised wire floors. Artificial light was provided for 16 hours per day and the ambient temperature was maintained in the comfort zone. The birds were given feed and water ad libitum to the assay period. The assay was initiated by fasting the birds for 24 hours, after which they were force-fed 30 g of the test ingredient. A group of control birds was fasted during this period and the excreta were collected to allow measurement of the endogenous amino acid production. Excreta were collected for a 48-hour period using a plastic tray placed under the cage. Excreta samples were lyophilized, weighed, ground and analyzed for dry matter and amino acids. The formula used in calculations was that reported by Parsons (1985a).

Multienzyme assay

The procedure for the multienzyme assay was described by Hsu et al. (1977). A sample of each of the ingredients used in the previous experiments was finely ground (60 mesh screen), and duplicate, 50 ml aqueous suspensions (6.25 mg protein/ml) were prepared using warm distilled water. These preparations were adjusted to an initial pH of 8 while stirring at 37°C in a water bath. Five ml of iced, multienzyme solution (1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase/ml) were added to the protein suspension. The drop in pH was recorded at 15 s, 1, 5 and 10 min using a Cole-Palmer Chemcadet Model 5984 pH meter equipped with a standard glass electrode.

Results and Discussion

Swine ileal digestibility

Values presented in Table 1 are true amino acid digestibility (TAAD) estimates (expressed as a percent) for the eight feedstuffs. These values are in good agreement with literature (Zebrowska, 1978; Tanksley et al., 1981; Sauer et al., 1982). Values for apparent digestibility were also calculated. Calculated values for apparent digestibility were lower than those for TAAD.

Lysine and threonine were, in general, less digestible than the other amino acids in corn and WHB. This same trend has been reported by Sauer et al. (1977) and Taverner et al. (1981b). Lysine digestibility in corn and WHB and threonine digestibility in WHB were significantly lower ($P \leq .01$) than in the other sources of protein with the exception of lysine in CSM and threonine in MBM 1. The true lysine digestibility measured for corn (59.9%) is in the range of values found in the literature. Published values range from apparent digestibility of 48% (Black and Davies, 1987) and 56.8% (Green et al., 1987) to true digestibility of 83% (Taverner et al., 1981b). The low values found in this experiment may be related to the low protein content (7.33%) of the corn used.

Precision-fed rooster assay

The TAAD values for the feedstuffs were calculated in the precision-fed assay. True lysine digestibility in CSM (71.82%) was significantly lower ($P \leq .01$) than in all the other feed sources. This percentage is intermediate to the ones presented by Nwokolo et al. (1976) and Anonymous (1988b). It is interesting that true lysine digestibility in CSM was the lowest value reported for an ingredient by Anonymous (1988b). In this experiment, it was not possible to detect any difference ($P \geq .01$) for true lysine digestibility among the other ingredients. True digestibility for threonine and tryptophan was higher ($P \leq .01$) in corn than in the other feedstuffs.

Average digestibilities for amino acids in CSM, PBP and MBM in this experiment were in general higher than those listed by Anonymous (1988b). However, values greater than ours have been reported by Nwokolo et al. (1976) for CSM and by Burgos et al. (1974) for PBP. Data from Hung and Kermorgant (1982) for MBM provide values similar to those obtained in our experiment. Values for true digestibility of lysine for the PBP used in this experiment were intermediate to those calculated by Herro et al. (1988) using either cecectomized or conventional roosters.

Multienzyme assay

Changes in pH were measured in the multienzyme assay system during a 10 min period. The 10 min reading was used to calculate the percent of pH drop in relation to casein. Incubation for less than 10 min is insufficient and for more than 10 min does not improve the assay according to Hsu et al. (1977). A low pH, for example that is found with casein, is indicative of extensive protein hydrolysis. The 10 min pH reading for each test ingredient was related to casein which for the purpose of this assay, was considered 100% digestible. The ratio was then expressed as a percent of enzyme hydrolysis relative to casein (ENZRC).

Table 1. True amino acid and crude protein digestibilities for eight feedstuffs based on swine ileal digesta collections*.

Amino acids	Ingredients*								
	Corn	WHB	SBM 1	SBM 2	CSM	PBP	MBM 1	MBM 2	SE*
Intake, g	1790	1351	1872	1899	1773	1902	1583	1601	105.79
Ala	79.91	63.35	81.63	81.97	69.35	80.87	80.23	81.00	1.80
Arg	94.43	94.95	96.49	96.11	92.78	89.81	88.18	89.63	1.51
Asp	73.89	67.56	85.38	85.12	78.04	62.60	69.73	69.62	2.17
Glu	84.53	85.72	88.72	88.29	85.22	77.55	73.54	75.02	1.49
Gly	67.76	55.69	77.88	78.73	69.91	78.99	80.81	82.83	3.07
His	78.53	73.90	86.47	87.13	79.26	77.44	74.46	76.58	1.66
Ile	78.96	72.48	87.49	87.14	73.62	80.60	74.65	75.36	2.22
Leu	83.33	67.31	84.22	83.71	72.11	78.59	74.44	75.92	2.20
Lys	59.92	60.00	86.07	85.43	59.72	78.15	75.86	76.52	2.02
M+C	86.17	75.93	91.83	90.91	82.99	81.70	79.23	77.87	2.33
Phe	84.51	78.14	87.95	87.72	84.06	80.07	78.28	79.02	1.80
Pro	79.71	92.21	88.59	89.50	82.16	80.65	83.33	78.93	2.54
Ser	78.40	71.74	85.77	85.57	77.51	75.74	72.16	74.34	1.66
Thr	71.69	59.56	82.90	81.54	71.50	74.15	69.11	72.65	2.52
Trp	68.39	73.25	82.79	86.37	73.93	61.51	60.82	64.31	4.39
Tyr	78.40	55.99	83.63	80.43	78.27	60.99	51.73	63.60	3.97
Val	79.26	72.69	85.98	85.41	76.93	78.87	76.40	76.70	2.02
CP*	78.97	74.98	84.01	85.73	73.91	76.61	75.95	77.26	1.69

*Values are means of three observations with one pig per observation.
*Abbreviations used in the table headings are: WHB = wheat bran; SBM 1 and SBM 2 = soybean meals 1 (normal) and 2 (heat treated); CSM = cottonseed meal; PBP = poultry by-product; MBM 1 and MBM 2 = meat and bone meals from two origins
*Pooled standard error of the means
*Least significant difference ($P \leq .01$)
*Crude Protein

Previously in this paper estimates of amino acid digestibility for several ingredients were obtained by ileal sampling procedures and precision-fed cecectomized rooster. The values for lysine, threonine and tryptophan digestibility are summarized in Table 2. In Table 3, values for the digestibility of lysine, threonine, and tryptophan are presented according to the different assays used for estimation.

The digestibilities obtained by the various methods (Table 2) were correlated for six ingredients. The corn and WHB exclusion from the data set improved the coefficients of correlation. When only the high-protein ingredients are considered, the estimates of lysine digestibility were well correlated ($r = .73$ to $.99$) among all the methods used. The "in vitro" multienzyme assay for protein hydrolysis was highly correlated with lysine digestibility and estimated by the two "in vivo" methods. This result agrees with the findings presented by Hsu et al. (1977) and Satterlee et al. (1977). Our data support the concept that the "in vitro" multienzyme method can be used to quickly estimate the utilizability of protein in high-protein ingredients.

Table 2. Summary of amino acid digestibility or availability values obtained for eight feedstuffs using four different methods.

Ingredient*	Lysine			Threonine		Tryptophan	
	ILEDL*	PREDL*	ENZRC*	ILEDTH*	PREDTH*	ILEDTP*	PREDTP*
Corn	59.92	95.66	74.29	71.69	109.03	68.39	101.80
WHB	60.00	85.83	76.77	59.56	90.48	73.25	92.54
SBM 1	86.07	92.94	85.11	82.90	91.05	82.79	92.28
SBM 2	85.43	91.64	84.04	81.54	89.56	86.37	94.52
CSM	59.72	71.82	79.96	71.50	81.92	73.93	84.33
PBP	78.15	84.15	82.45	74.15	84.38	61.51	85.86
MBM 1	75.86	85.04	82.98	69.11	84.18	60.82	84.39
MBM 2	76.52	85.52	82.62	72.65	85.36	64.31	82.53

*Abbreviations used in the table headings are: WHB = wheat bran; SBM 1 and SBM 2 = soybean meals 1 (normal) and 2 (heat treated); CSM = cottonseed meal; PBP = poultry by-product; MBM 1 and MBM 2 = meat and bone meals from two origins
*Ileal digestibility of (L) lysine, (TH) threonine and (TP) tryptophan.
*Precision-fed digestibility of lysine, threonine and tryptophan.
*Multienzyme hydrolysis of the ingredient in relation to casein.

Table 3. Pooled amino acid digestibility or availability values for each of the three methods investigated*.

Digestibility	Ileal digestibility	Precision-fed	Multienzyme assay	SE*
Lysine*	72.71	86.58	81.03	3.73
Threonine	72.89	89.50	81.03	2.44
Tryptophan	71.42	89.78	81.03	2.28

*Values are means of eight different ingredients.
*Pooled standard error of the means.
*Least square means.

Conclusions

1 -- The methods used produced different responses for the amino acids studied where the values for ileal digestibility were the lowest and the precision-fed assay the highest.

2 -- When corn and wheat bran were excluded from the analysis, the coefficients of correlations improved indicating that all methods were associated for lysine.

3 -- The multienzyme assay presented high correlation with the other methods.

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THE EFFECTS OF DIETARY CARBOHYDRATES ON MICROBIAL GROWTH AS DETERMINED BY CONTINUOUS IN VITRO-INCUBATIONS OF HINDGUT CONTENTS OF PIGS

G. Breves[1] & J. Dreyer

Institute of Animal Nutrition, Federal Agricultural Research Centre, Braunschweig, FRG

Abstract

The "colon simulation technique" was applied as a continuous in vitro-model for studying basic mechanisms of microbial hindgut metabolism in pigs. Gauze filtrated caecal fluid was used to start each incubation experiment and caecal particles were applied as fermentable substrate. Each experiment lasted for 16 days including an equilibration period of 8 days. The experiments were done on three diets with decreasing cellulose and corresponding increases in hemicellulose concentrations. The highest VFA production rate was determined when approximately 70% of fibrous carbohydrates consisted of cellulose. Since the composition of the diet had no effect on microbial protein synthesis the lowest efficiancy of microbial growth was calculated for the high cellulose diet.
Keywords: Hindgut, microbial growth, efficiancy, dietary carbohydrates.

Introduction

It has been shown from studies on rumen metabolism that the efficiancy of microbial growth may substantially be altered by changing the composition of the diet (Stern et al.1978; Rohr et al. 1989). In order to obtain a more detailed information about basic mechanisms of microbial hindgut metabolism in pigs it was the aim of the present paper to study quantitative parameters of microbial growth in relation to the proportion of cellulose and hemicellulose in three different experimental diets. For these studies the "colon simulation technique" was used as a continuous incubation procedure. This technique has been proved as a sensitive and reproducible method for studying microbial metabolism in the large intestines (Breves et al. 1990).

Materials and Methods

The experiments were performed on three diets which were characterized by increasing the cellulose content from 44 to 109 g/kg dry matter and correspondingly decreasing

1. Present address: Institute of Veterinary Physiology, Gießen, FRG

hemicellulose concentrations from 152 to 50 g/kg. These changes were achieved by combining different grain components, field beans, maniok, cellulose, coconut and fish meal at varying proportions. At least three weeks were allowed for adaption to each diet and pigs with a body weight between 100 and 300 kg were used as donor animals. For each in vitro-experiment five incubation vessels were run simultaneously and the equilibration period of 8 days was followed by 8 sampling days for measuring pH, production of volatile fatty acids (VFA), organic matter (OM) digestibility and microbial protein synthesis. In all experiments a five-fold daily fluid turnover was maintained which was equivalent to a mean fluid retention time of 4.8h. Particle retention time was adjusted to 24h.

Results and Discussion

On diet 1 (109 g cellulose and 50 g hemicellulose /kg DM) the mean VFA production was more than twice as high with corresponding decreases in pH and increases in organic matter digestibility as compared to the other two diets. The ratio between VFA production and digestion of organic matter ranged between 6 and 14 mmol VFA/g digested organic matter with the highest efficiency of VFA production in diet 1. Changing the cellulose/hemicellulose proportions had no effect on microbial protein synthesis which ranged between 225 and 269 mg/d. Thus the efficiency of microbial growth expressed as assimilation of microbial N / digested organic matter (mg/g) was lowest in diet 1. These results confirm data which were obtained from studies on rumen metabolism comparing the effects of cellulose and starch on efficiency of microbial growth (Stern et al. 1978). The results indicate that the ratio and possibly the origin of dietary fibrous carbohydrates is a major factor for regulation of microbial growth efficiency in the large intestines. Since the quantitative growth parameters obtained from the present study were in a similar range as it has been described for the rumen it may be concluded that the major basic mechanisms of microbial metabolism in the large intestines are comparable to those in the rumen.

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IMPACT OF EXOCRINE PANCREATIC ADAPTATION ON IN VITRO PROTEIN DIGESTIBILITY

P. Valette[1], H. Malouin[2], T. Corring[1] and L. Savoie[2]

[1]Laboratoire d'Ecologie et de Physiologie du Système digestif, INRA, Jouy en Josas, France
[2]Département de Nutrition Humaine et de Consommation, Université Laval, Québec, Canada

Abstract

An in vitro enzymatic method was used to study kinetics of digestion of casein and rapeseed proteins. After a predigestion with pepsin, the protein substrates were submitted to a 24-hours hydrolysis either with pancreatin or pancreatic juices of pigs adapted either to casein or rapeseed diet. After 3, 6 and 24 hours of digestion, dialysates were collected and analysed for content of nitrogen and amino acids. For a long term hydrolysis (24 hrs), overall digestibility of both substrates was not affected by the composition of pancreatic enzymes mixtures. However, in the beginning of hydrolysis kinetics, a significant effect of pancreatic juices (mainly "casein pancreatic juice") was observed on the rapeseed nitrogen digestibility. During the first hours of digestion, individual amino acid digestibility was generally higher when "casein pancreatic juice" was used for hydrolysis. It is concluded that enzyme mixture used for in vitro hydrolysis do not influence nitrogen and amino acid digestibilities for a long-term digestion. But an effect of the enzymatic source appears at the beginning of hydrolysis kinetics and is markedly dependent on the nature of the protein tested.
Key words :in vitro protein digestibility, casein, rapeseed, pancreatic juice

Introduction

In the pig fitted with permanent cannulae in the pancreatic duct, the feeding of a meal whose protein component was either casein or rapeseed concentrate markedly modified the composition of pancreatic enzyme secretion (Valette et al., 1990).

The purpose of the present work was to study the impact of this pancreatic response on the nitrogen and amino acid digestibilities of casein and rapeseed concentrate. After a predigestion with pepsin, casein and rapeseed proteins were hydrolysed in vitro, with the digestion cell technique (Savoie & Gauthier, 1986) in presence of the pancreatic juices of pigs adapted either to casein or rapeseed diet. The hydrolysis kinetics were compared to standard digestion with pancreatin.

Materials and Methods

Casein (83.6 g N x 6.25/100 g DM) and rapeseed concentrate (52.9 g N x 6.25/100 g DM) were used for the different in vitro assays. Pancreatic enzymes (freeze-dried) were obtained from fistulated pigs adapted fifteen days to a diet containing either casein (casein pancreatic juice) or rapeseed concentrate (rapeseed pancreatic juice) as the sole source of protein (12 g/100 g DM diet). Before use, the enzyme mixture (50 mg of protein) was activated with 1 ml of trypsin in tris Ca^{++} buffer (10 ml), pH 7.9, for 24 hrs at 4°C. Pancreatin was used as such.

After 30 minutes of pepsic digestion, dialysates were poured into the dialysis tube (1000 dalton MWCO, Spectra Por®6, Spectrum Medical Industries, Inc., Los Angeles, CA) of the digestion cell. Digestion was started by adding 1 ml of pancreatin (10 mg/ml phosphate buffer) or 2 ml of pre-activated pancreatic juices (5 mg/ml). Digested material diffusing through the membrane was collected by a circulating (1.6 ml/min) phosphate buffer (0.01 M, pH 7.5). Digestion lasted for 24 hours with aliquot sampling after 3, 6, and 24 hrs. Nitrogen and amino acids were determined in the following pooled samples: 0-3, 0-6 and 0-24 hours.

Results

Table 1 . In vitro nitrogen digestibility (%) of casein and rapeseed proteins with pancreatin (P), casein (C) and rapeseed (R) pancreatic juices.

Time of digestion (hours)	Casein*			Rapeseed*		
	P	C	R	P	R	C
3	$28,2^{ab1}$	$32,4^{a}$	$28,3^{ab}$	$18,6^{c}$	$24,0^{bc}$	$26,7^{ab}$
6	$50,8^{ab}$	$54,7^{a}$	$48,6^{ab}$	$39,4^{c}$	$44,4^{bc}$	$50,2^{ab}$
24	$90,9^{a}$	$89,5^{a}$	$84,6^{a}$	$86,2^{a}$	$82,5^{a}$	$85,1^{a}$

* protein sources were previously hydrolysed with pepsin at pH 1,9 for 30 minutes
1 cumulative values means of four assays. Within a lign, values affected by the same superscript letter are not significantly different ($P < 0,05$)

Table 1 showed the in vitro nitrogen digestibility of casein and rapeseed concentrate with various pancreatic mixtures. In the first 6 hrs, casein nitrogen digestibility with commercial pancreatin was significantly ($p<0.05$) higher than that of rapeseed concentrate. After 24 hrs, the differences levelled. With casein as substrate, nitrogen digestibility was not significantly affected whatever the enzymatic source used. With rapeseed concentrate as substrate, the use of rapeseed pancreatic juice produced a small but not significant rise in nitrogen digestibility during the two first intervals of digestion. On the contrary, rapeseed nitrogen digestibility was significantly increased with the casein pancreatic juice, during the first six hours of digestion, values being equivalent to those obtained with casein hydrolysed by rapeseed pancreatic juice.

As showed in table 2, after three hours of in vitro digestion, the small increase in casein nitrogen digestibility elicited by casein pancreatic juice was mainly due to a more important release of arginine and leucine. Rapeseed amino acids digestibility was enhanced by the use of pancreatic juices, this effect being more important with casein pancreatic juice. Significant increases were observed with arginine, histidine, leucine, lysine and methionine.

As the digestion proceed (after 6 hrs), casein amino acids digestibility was not influenced by the enzyme mixture used for hydrolysis. With rapeseed as substrate, the increase elicited by casein pancreatic juice was still significant for the same amino acids previously cited. The rise produced by rapeseed pancreatic juice was perceptible, but closely equally distributed among amino acids.

After 24 hrs of hydrolysis, when the protein sources were almost completely digested, the differences in casein and rapeseed amino acids digestibility observed during the first two intervals between enzymatic sources were nearly abolished.

Table 2 . In vitro amino acid digestibility (%) of casein and rapeseed proteins after 3 hrs of digestion with pancreatin (P), casein (C) and rapeseed (R) pancreatic juices.

Amino acids	Casein*			Rapeseed*		
	P	C	R	P	R	C
Arginine	52^{b1}	59^{a}	45^{c}	19^{e}	34^{d}	40^{d}
Histidine	28^{ab}	35^{a}	30^{ab}	21^{c}	27^{bc}	31^{ab}
Leucine	35^{b}	41^{a}	36^{ab}	22^{d}	27^{cd}	30^{bc}
Lysine	43^{a}	49^{a}	44^{a}	18^{c}	30^{b}	35^{b}
Methionine	30^{ab}	34^{a}	29^{ab}	21^{c}	27^{b}	29^{ab}

* and 1, see table 1

Discussion

In this work, we observed that total nitrogen digestibility was not significantly different after 24 hrs of an in vitro digestion 1) between values obtained with the three enzyme mixtures for a same protein substrate, 2) between protein substrates wathever the enzymatic sources used for hydrolysis. However, the enzymatic sources and the nature of protein substrate led to major differences during the first hours of digestion.

For enzymatic sources, it appeared a pancreatic juice effect and particularly a better efficiency of casein pancreatic juice on the rapeseed nitrogen digestibility. Our results did not allow to explain this observation. A plausible hypothesis is the relationship which can exist between the hydrolysis potency and the proteolytic enzyme equipment of enzyme mixtures. However, only specific activity of carboxypeptidase A is more important in the casein pancreatic juice than in other enzymatic sources.

Concerning the nature of protein substrate we observed that nitrogen release was markedly enhanced in casein compared to rapeseed substrate during the first 6 hrs of digestion, whatever the enzymatic source used. Same results were obtained by Savoie et al. (1988) and these differences may be the consequence of the pre-digestion with pepsin. Pepsin could produce destabilization of the micellar structure of casein by a specific breakdown of its kappa fraction (Pélissier, 1984). It may not have the same effect on the more complex and chemically stable structure of rapeseed globulins (Gray & Cooper, 1971). The release of digestion products was thus delayed with this latter protein as compared to casein substrate. The purity of the protein was also expected to be a factor modulating the digestibility since the non-proteic components of a foodstuff are known to interfere with protein digestion (Silano, 1976). In our experiment, The protein content was weaker in rapeseed concentrate than in casein (52,9 g/100 g DM and 83,6 g/100 g DM respectively) since rapeseed contained some non-proteic components such as fibers or tannins.

For both substrates, the amino acid digestibility was not influenced by the nature of enzyme mixture for a long-term in vitro digestion (24 hrs). But, the effect of the enzymatic source appeared primarily in the beginning of the digestion. With casein as substrate, the digestibility of the whole amino acids was improved by the use of casein pancreatic juice during the first 6 hrs of the digestion. In the same way, rapeseed amino acids release was more important with pancreatic juices than with pancreatin and the casein pancreatic juice had the best potency. Moreover, we noticed that the use of pancreatic juices modified the relative proportion of certain

amino acids in the dialysate; for instance, arginine and lysine proportions, measured after 3 or 6 hrs of digestion, were clearly enhanced. The relative digestibility index (RDI) calculation (amino acid digestibility/nitrogen digestibility), which reflects the release of one amino acid in comparison with the whole amino acids, allowed to illustrate this phenomenon. Indeed, after 3 hrs of digestion, RDI values of lysine and arginine were 0.97 and 1.02 respectively when using pancreatin, 1.25 and 1.50 with rapeseed pancreatic juice and finally 1.31 and 1.42 with casein pancreatic juice. When the digestion was purshased to 6 hrs, the same ratio was obtained. Consequently, arginine and lysine were more slowly released with pancreatin comparatively to the use of pancreatic juices.

The improvement of certain amino acid digestibility led us to suspect a major effect due to the enzyme specificity. For instance, extensive liberation of lysine and arginine with pancreatic juices would suppose a greater trypsic activity in these enzyme mixtures. But, specific activity of trypsin was higher in pancreatin than in pancreatic juices. In the same way, the greater specific activities of carboxypeptidase A and chymotrypsin in the casein pancreatic juice, comparatively to the pancreatin did not result in a better release of target amino acids of these enzymes (tyrosine and phenylalanine). Finally, it is difficult to anticipate for the specific contribution of each enzyme. According to Gertler et al. (1980), the hydrolysis efficiency of one enzyme was greatly dependent on the simultaneous action of the other proteases.

In the present study, we observed that the nature of enzyme mixture can influence casein and rapeseed hydrolysis in terms of nitrogen and amino acids digestibility. But our results showed that variations due to enzymatic source were observed only during the first hours of the in vitro digestion, results obtained after a long-term proteolysis being independent on the enzyme mixture.

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A COMPARISON OF TRUE DIGESTIBILITY FOR POULTRY AND APPARENT ILEAL DIGESTIBILITY FOR SWINE. A CLASSICAL IN VITRO METHOD AND NIR SPECTROPHOTOMETRY FOR DETERMINING AMINO ACID DIGESTIBILITY

M.D. Harrison[1], M.R.M. Ballard[2], R.A. Barclay[3], M.E. Jackson[4] & H.L. Stilborn[2].

Heartland Lysine, Inc., Chicago, Illinois, U.S.

Abstract

Nineteen samples of blood meal commonly used commercially in North America were assessed for True Amino Acid Digestibility (TD) in poultry and for Apparent Ileal Amino Acid Digestibility (AD) in Swine. The samples were also assessed for in vitro digestibility via a multienzyme assay and NIR spectrophotometry. Lysine digestibility for both swine and poultry was correlated with digestibility of arginine, threonine, methionine and tryptophan within species ($P<.01$) but not correlated among species ($P>.1$). The multienzyme in vitro method was correlated with AD of lysine ($P<.001$) but not with TD ($P>.18$). NIR calibration equations were developed for total lysine, AD lysine and TD lysine. Optimum wavelengths were different for each of the calibrations developed. These data indicate the use of NIR for predicting digestibility of lysine for swine and poultry may be feasible. However, larger sample sets will be required to generate calibrations with application to the feed industry.

Introduction

Providing amino acids to an animal in a form which optimizes performance is one of the major goals of every nutritionist. It is widely accepted that the value of feed ingredients for supplying dietary protein is dependent upon both the content and digestibility of their amino acids. In order to formulate a diet, the nutritionist must assume that dietary amino acids are completely utilized or must adjust the amino acid minimums to allow for something less than 100% utilization. Published values for digestibility of amino acids in swine and poultry are widely available which offer an excellent starting point. However, the nutritionist must still account for the amino acid digestibility in each batch of feed ingredient which is mixed into a diet to have confidence that the nutrient needs of the target animal have been met for specific productive purposes.

A tremendous amount of research has been conducted to estimate the digestibility of amino acids in feed ingredients. Comparisons of methodology and compilation of averages have been the focus of these research efforts for a number of years. In swine the question of fecal vs ileal digestibility has been reviewed (den Hartog et al. 1987; Sauer, 1977). Additionally the issue of apparent vs true digestibility has also been a traditional subject of discussion (Sauer & Ozimek, 1985; Green, 1987; Sauer & De Lange, 1988). In poultry the principal studies have focused upon a standardized methodology and whether intact or cecectomized cockerels offer an advantage in the assay (Sibbald, 1987; Parsons, 1986).

1. Present address: A.E. Staley Mfg. Co., Decatur, IL.
2. Present address: Heartland Lysine Inc., Chicago, IL.
3. Present address: Washington University, St. Louis, MO.
4. Present address: Continental Grain Co., Chicago, IL.

With the compilation and recent publication of mean values for amino acid digestibility in swine and poultry, the focus in research has shifted to the ability to rapidly predict amino acid digestibility. Two in vivo assays were compared with each other together with two in vitro methods to investigate the ability of the more rapid and less expensive in vitro assays to predict the in vivo results.

Materials and Methods

Twenty samples of blood meal were obtained from commercial feed manufacturers and evaluated for amino acid digestibility in poultry and swine. The samples were selected based upon commercial availability and encompassed the principal blood meal processing methods currently employed in North America. Subsamples were evaluated for AD and TD. All feed, ileal and excreta samples were evaluated for amino acid content at the Heartland Lysine laboratory in Eddyville, Iowa.

In Vivo Assays

Swine Digestibility

Eight crossbred barrows initially weighing 40 kg were surgically fitted with T-cannulae approximately 4 to 5 cm anterior to the ileocecal valve. Following a 14 day recovery period, the experiments were initiated. Pigs were housed in an environmentally controlled room in metabolism cages. The pigs were put on a seven day collection period consisting of a four-day adjustment followed by three day ileal digesta collection. Diets were fed at a constant level based upon intake during the adjustment period and were presented twice a day at 12-hour intervals. Water was offered free choice during the entire collection period. Diets were supplemented with minerals and vitamins to meet or exceed NRC requirements, and .25% chromic oxide was added as an indigestible marker. Apparent ileal digestibility was determined by the classical difference method.

Poultry Digestibility

Eighty adult male cockerels were cecectomized and maintained as a population for ingredient evaluation. All birds were fed the same basal laying ration for 14 days prior to initiation of the study and were housed under continuous light at 24°C. The birds were randomly allotted to 16 treatments, each having five replicates. Sixty grams of test material was tube fed following a 48-hour feed deprivation period. Water was provided ad libitum throughout the test period. Excreta were collected via colostomy bags for 48 hours following feeding. A separate 48-hour collection was conducted for fasted birds provided only water in order to assess endogenous losses. True amino acid digestibility was calculated as described by Sibbald (1986).

In Vitro Assays

Multienzyme Assay

The procedure used for prediction of digestibility was described by Satterlee et al (1982). Ten mg nitrogen equivalent of each sample was analyzed in duplicate for a pH drop in a buffered solution (initial pH 8.0 +/- .03) after exposure to a series of proteolytic enzymes (Satterlee et al, 1982). The drop in pH after a 20-minute digestion was corrected with a standard (sodium caseinate) and compared with in vivo lysine digestibility results.

NIR Assay

Approximately 10g of each sample was ground in a Udy Cyclone mill and packed into a quartz window sample cup. NIR spectra were obtained on an NIR Systems Model 5000 spectrophotometer (Perstorp Analytical Herndon, VA). Spectra were recorded as log 1/R and transformed to the first derivative of the log 1/R signal for analysis. Optimum wavelengths for prediction of lysine, AD lysine and TD lysine were determined by multiple linear regression analysis of first derivative log 1/R as described by Norris and Williams (1983). Due to insufficient sample number, a validation set was not available to verify accuracy of the calibration. Results of NIR predictions for lysine, AD lysine and TD lysine are comparisons made within the calibration set.

Results and Discussion

In Vivo Digestibility

Mean digestibility for 19 blood meals of selected essential amino acids for swine and poultry are presented in Table 1. These values are in agreement with published values (HLI 1990a,b). Apparent digestibility of methionine and tryptophan was lower and more variable than other amino acids. Correlation of lysine digestibility with the other selected amino acids is presented in Tables 2 & 3. Lysine digestibility was well correlated within species ($P<.001$) with the digestibility of arginine, methionine, threonine and tryptophan. However, there was no correlation ($P>.1$) between observed AD values for swine and TAAA values for poultry. This is in contrast to the relatively good correlations reported by Jackson (1989). The data reported by Jackson (1989) was compiled across ingredients with a maximum of four observations for any one ingredient. It is possible that correlations across species may differ due to ingredient. Regression analyses were conducted comparing AD lysine with the other amino acids presented. This simple model accounted for 50 to 80% of the variation in this prediction as estimated by R2 depending upon the amino acid studied. Similar values were observed for TD in poultry with simple regression of TD lysine on TD of the other amino acids accounting for 54 to 95% of the variation as assessed by the coefficient of determination.

Table 1. Digestibility of selected amino acids in blood meal for swine and poultry.

	Swine (AD)[1]			Poultry (TAAA)		
	coeff	n	SBM	coeff	n	SEM[2]
Arginine	78	19	3.3	87	15	3.4
Lysine	76	19	3.5	86	14	3.1
Threonine	73	19	3.6	85	15	2.7
Methionine	68	16	4.4	85	15	3.6
Tryptophan	66	11	4.7	86	11	2.1

* Abbreviations: Coeff = Digestion coefficient %; n = number of observations; SEM = standard error of mean.

[1] Mean of two observations with one pig per observation. Apparent Ileal Digestibility.

[2] Samples pooled across five replicate birds for analyses. True Amino Acid Digestibility.

Table 2. Correlation of swine ileal lysine digestibility with digestibility of selected other amino acids.

Lys	Arg	Met	Thr	Trp
cc*	.89	.73	.86	.83
p value	.0001	.0012	.0001	.0001
n*	19	16	19	19

* cc = Pearson correlation coefficients.
* n = number of observations.

Table 3. Correlation of poultry TAAA lysine with selected other amino acids.

Lys	Arg	Met	Thr	Trp
cc*	.98	.98	.96	.74
p value	.0001	.0001	.0001	.01
n*	14	14	14	11

* cc = Pearson correlation coefficients.
* n = number of observations.

In Vitro Assays

Correlation of the multienzyme assay (Satterlee et al., 1982) with AD and TD is presented in Table 4. The in vitro assay was well correlated with AD for the amino acids studied. There was a very poor correlation between this multienzyme assay and the TD values for poultry. The values reported for AD agree generally with those reported in the literature (Babinszky et al., 1987; Dierick et al., 1985; Babinszky et al., 1990). Bellavar (1989) reported stronger AD correlations than were observed in this study using a similar assay on a mixed matrix of ingredients. However, no previous research has accumulated as many observations for a single ingredient thereby removing the ingredient effect on the assay.

NIR predictions of the AD and TD values are presented in Table 5. The related statistics indicate that NIR is at least as good as the multienzyme method tested in this study. In general a preliminary calibration on most NIR instruments requires at least 40 samples. The size of the data set used for this calibration lends itself to overfitting of the model by the statistical software used for the calibration development. The cost of generating this number of samples is prohibitive at this time, as the in vivo assays are expensive. The data here serve to illustrate the potential for this technology to be applied in the area of animal nutrition.

Table 4. Correlation of in vitro pH change with digestibility of selected amino acids for swine and poultry.

	Arg	Lys	Met	Thr	Trp
Swine					
cc*	-.71	-.72	-.58	-.62	-.72
p value	.0007	.0005	.02	.005	.0005
n*	19	19	16	19	19
Poultry					
cc	-.43	-.38	-.47	-.48	-.16
p value	.10	.18	.08	.07	.63
n	15	14	15	15	11

* cc = Pearson correlation coefficients.
* n = number of observations.

Table 5. NIR prediction of AD & TD for blood meal.

	Swine			Poultry		
Sample	In Vivo	NIR	Residual	In Vivo	NIR	Residual
1	93	88	5	96	100	-4
2	92	91	1	87	91	-4
3	95	93	2	48	55	-7
4	86	73	13	65	72	-7
5	95	99	- 4	92	80	12
6	47	46	1	16	82	4
7	65	59	6	88	82	6
8	72	73	- 1	95	100	-5
9	71	74	- 3	82	79	-3
10	73	81	- 8	89	92	-3
11	86	73	13	93	82	11
12	93	87	6	82	84	-2
13	64	64	0	92	88	4
14	46	56	-10	81	85	-4
15	89	79	10	91	93	-2
16	67	80	-13			
17	78	80	- 2			
18	64	69	- 5			
19	70	81	-11			

Swine: R^2=.75, SEP=7.43, N=19 Poultry: R^2=.77, SEP=.27, N=15

Conclusions

The digestibility of selected amino acids in blood meal for swine and poultry are related to the lysine digestibility within species. There appears to be no relationship between AD and TD in blood meal. The multienzyme assay studied was well correlated with AD for all amino acids studied. This method offers possibilities toward developing a reasonably rapid assay for AD in swine although its use appears limited to differentiating between samples which vary substantially. Potentially it could be used as a screening assay for ingredient quality rather than an accurate predictive method.

NIR spectrophotometry has the potential to be developed into a reliable method for predicting AD and TD. Calibration development is limited by the number of in vivo assays available for scanning. The major barrier to further development of this method is the cost of producing the in vivo observations. Refinements to this data set and accumulation of additional samples is necessary before either method becomes usable by the feed industry.

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NEAR INFRARED REFLECTANCE (NIR) SPECTROSCOPY TO ESTIMATE THE APPARENT ILEAL DIGESTIBILITY OF PROTEIN IN FEEDSTUFFS

P. van Leeuwen[1)], M.W.A. Verstegen[2)], H.J. van Lonkhuijsen[3)], G.J.M. van Kempen.[1)]

[1)]TNO-Institute for Animal Nutrition and Physiology (ILOB), P.O.Box 15, 6700 AA Wagenigen, The Netherlands.

[2)]Department for Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wagenigen, The Netherlands.

[3)]Biochemical and Physical Chemistry Department, TNO Biotechnology and Chemistry Institute, P.O.Box 360, 3700 AJ Zeist, The Netherlands.

Abstract

In present study the applicability of NIR analyses for ileal digestibility in feedstuffs was evaluated. A calibration of the NIR was made based on a wide variety of feedstuffs in which the ileal digestibility had been determined in the classical way by chyme collection.

With an InfrAlyser 400 the absorbance at 19 different wavelengths was determined. The calibration developed for digestibility contained 7 wavelengths, 1759 nm, 1772 nm, 1818 nm, 2139 nm, 2180 nm, 2310 nm, and 2336 nm, respectively. The correlation coefficient (R) and residual standard deviation after regression of in vivo on absorption at the 7 different wavelengths were 0.90 and 4.3 units of digestibility respectively.

Introduction

The nutritional value of protein from feedstuffs depends on, among other, the amount of amino acids that is digested and absorbed in the small intestine (Zebrowska., 1973; Dierick et al., 1987). For that reason the apparent ileal digestibility is an important parameter to evaluate the nutritional value for protein in feedstuffs. Classical determinations of the digestibility are expensive and time consuming. They require large amounts of feed and involve considerable expenditure of equipment and workers (Sauer et al., 1989). The development of alternative techniques therefore is a current topic of research.

An alternative to the classical digestibility experiments is the Mobile Nylon Bag Technique (MNBT) (Sauer et al., 1989). With this technique it is possible to predict the faecal digestibility of protein in feedstuffs. This technique has been altered to measure the ileal digestibility of protein (van Leeuwen et al., 1990).

Recent reports indicate that also the Near Infrared reflectance (NIR) analyses may be an alternative for measurement of the ileal digestibility of protein (Maes, I., 1990; Personal communication , Gives et al., 1990). The objective of the present study was to evaluate the applicability of NIR analyses for ileal digestibility in feedstuffs. In a pilot study a calibration of NIR was made based on a wide variety of feedstuffs. The ileal digestibility of these feedstuffs had been determined in the classical way by chyme collection.

Materials and Methods

a Principle of NIR analysis

In feed industry the NIR spectroscopy is a rapid technique for analysing contents of nutrients in feedstuffs (van Lonkhuijsen and Jansen, 1987). The principle of the technique is that compounds with similar chemical groupings absorb IR radiation at characteristic wavelengths. This is caused by absorption of energy in fixed amounts by a molecule at the natural vibration frequencies of oscillating chemical bonds between atoms in the molecule. The frequency or wavelength of this vibration is characteristic for the nature and the environment of the chemical bond which is vibrating. There are a number of possible vibrations of chemical groupings and each of these will have its own characteristic frequency. This gives a specific IR absorbtion as a kind of fingerprint for that grouping. In the NIR spectrum additional weaker absorption bands called overtones occur.

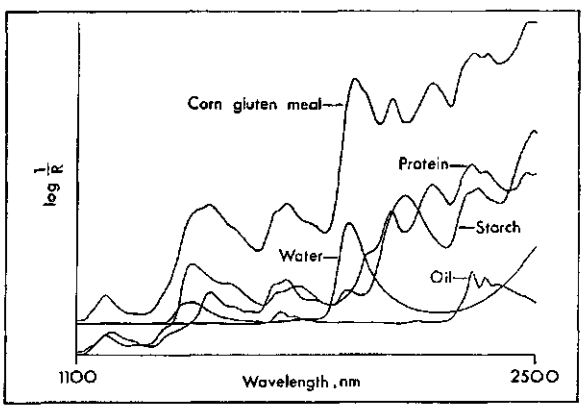

Figure 1. NIR reflectance spectra of some nutrients and water from a feedstuff (Osborne, 1985).

In Figure 1 the NIR spectra of water and some nutrients from a corn gluten feed are given. Each nutrient will have characteristic bands but there is overlap in bands (Figure 1) between nutrients. This overlap indicates that the whole spectrum is important and the idendification and quantification requires calibration of an unknown sample against samples with a known composition. (Osborne and Fearn, 1985,1986).
Complex parameters like digestibility are regressed on multiple absorbtion wavelengths. These relations however have to be known and quantified as a kind of calibration. This is based on a reference technique, which in this study is the classical determination. For this calibration, the choice of samples is crucial. The calibration curve should be made of good representatives of a class of feedstuffs to which both the classical ileal determination and the NIR analyses have been made. Then routine measurements with NIR can be made for samples falling in the same catagory of feedstuffs as the series with which the reference line has been made.

b Procedure of NIR analysis and NIR apparatus

The samples were ground with a cyclomill (Retsch) through a 1.0 mm mesh screen. The grinded samples were homogenized and loaded into a sample cup. The sample cup was placed on a drawer of the NIR instrument. Closing the drawer starts the process in which the instrument analyses the sample using near infrared radiation. The reflected radiation is captured by a detector, amplified and converted by the instruments microprocessor. A calibration is performed with a sample set consisting of a wide variety of samples from feedstuffs of which the ileal digestibility already is determined in the classical way.

With a standard InfrAlyser 400 (Bran + Luebbe, Hamburg, Germany) the reflectance at 19 wavelengths in range of 1200 to 2400 nm were registered. With the software package a calibration was made using the "best set" method.

c Samples for calibration

The calibration set consisted of 45 samples of different feedstuffs. In the set were grains, byproducts from grains, legume seeds, residues after solvent extraction, proteins from animal origin and alfalfa meal (Table 1). Samples of maize gluten feed were not included in this calibration.

The apparent ileal digestibilities of protein from the feedstuffs low in protein (< 12 %) were determined directly. The apparent digestibility of the other feedstuffs were determined with the difference method as described by van Leeuwen et. al (1987). The in vivo experiments were carried out with castrated male pigs from the Dutch Landrace x Great Yorkshire breed. The apparent digestibility of each feedstuff was determined with four animals with an initial live weight of 40 kg.

Results and Discussion

Digestibility is a complex parameter, influenced by different components. Consequently different wavelengths (filters) will be related to digestibility. Therefore the calibration of the digestibility was carried out with 19 filters, of which eventually 7 were chosen by the calibration software.

The calibration developed for digestibility contains 7 filters (figure 2, F02=1759 nm, F04= 1772 nm, F09= 1818 nm, F10= 2139 nm, F12= 2180 nm, F15= 2310 nm and F18= 2336 nm). This combination was assumed to be acceptable for a complex parameter. The correlation coefficient (R) and residual standard deviation were 0.90 and 4.3 units of digestibility respectively. These parameters indicate that NIR can be used as an indicative tool for the prediction of digestibility.

In Table 1 and Figure 2 the deviations between the apparent digestibility coefficients of crude protein (%) determined in in vivo experiments and the apparent digestibility coefficients of crude protein (%) predicted by NIR are given.

The 45 feedstuffs were divided in six groups: grains (I), byproducts from grains (II), legume seeds (III), residues from solvent extraction (IV) and proteins from animal origin (V) and one sample of a crude fibre rich product (VI). The mean digestibility values of the different groups of feedstuffs were nearly identical. Within the groups the results have been compared by use of the rank correlation test described by Spearman (de Jonge, 1963). The rank correlation of the predicted digestibilities

Table 1. Samples of the feedstuffs used for the calibration.

	Batch. No.	DC-CP (%) determined by in vivo experiments (a)	DC-CP (%) predicted by NIR (calibration) (b)	Deviation in vivo minus NIR (a-b)
Grains (I) and byproducts from grains (II),				
Maize	1	70	69	1
	2	60	71	-11
	3	70	66	4
	4	70	74	-4
	5	65	66	-1
	6	68	67	1
Barley	1	77	74	3
Mean DC-CP grains (I)		69	70	
Byproducts from wheat	1	89	81	8
	2	80	76	4
	3	77	73	4
	4	73	73	0
Byproducts from maize	1	71	71	0
	2	61	69	-8
	3	71	65	6
Mean DC-CP grain byproducts (II)		75	73	
Legume seeds (III),				
Vicia faba beans	1	73	73	0
	2	74	75	-1
	3	85	78	7
	4	69	73	-4
Peas	1	74	76	-2
	2	72	76	-4
	3	72	76	-4
	4	76	74	2
	5	70	70	0
	6	76	73	3
Lupins	1	82	85	-3
Mean DC-CP legume seeds (III)		75	75	
Residues after solvent extraction (IV),				
Soyabeanmeal	1	43	52	-9
	2	80	81	-1
	3	77	74	3
	4	75	81	-6
	5	83	79	4
	6	77	81	-4
	7	71	74	-3
Sunflower meal	1	76	71	5
	2	74	71	3
Rice bran	1	57	64	-7
Rape seed	1	74	73	1
Coconut meal	1	50	49	1
Mean DC-CP residues after solvent extraction (IV)		70	71	
Proteins from animal origin (V),				
Meat and bone meal	1	73	71	2
	2	70	80	-10
	3	74	75	-1
Fish meal	1	76	70	6
	2	82	74	8
Feather meal	1	57	60	-3
Caseine	1	97	93	4
Mean DC-CP proteins from animal origin (V)		76	75	
Crude fibre rich products (VI)				
Alfalfa meal	1	47	45	2

DC-CP = Apparent ileal digestibility coefficients of crude protein.

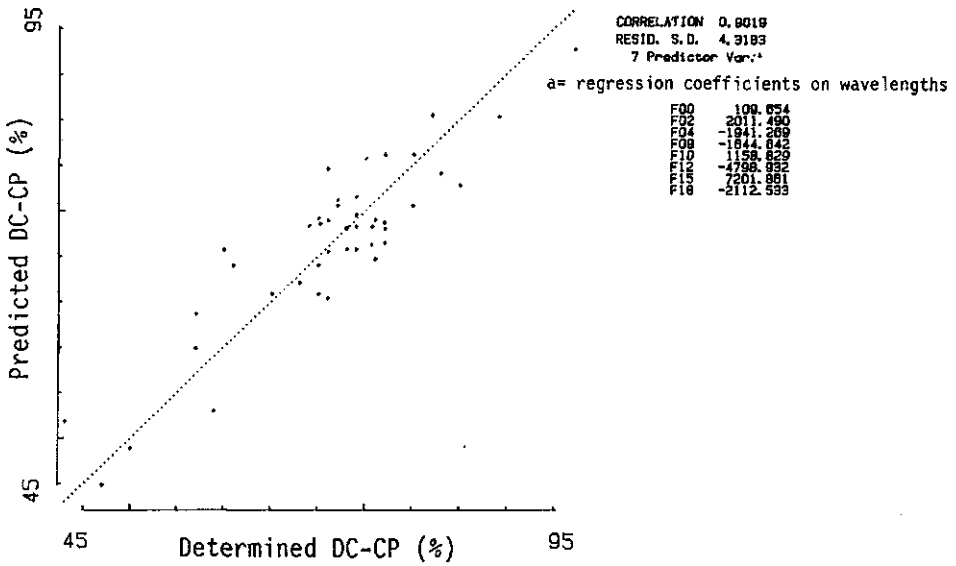

Figure 2. Relation between predicted (y axis) and determined (x axis) apparent ileal digestibility of crude protein (DC-CP). The predicted value has been calculated using the formula:
y = a1 * Foo + a2 * F02 +..... a8 * F18;
with F00 = 1; F02.....F18 = refectances at different wavelengths.
a1....a8 = regression coefficients on wavelengths

and the digestibilities from in vivo experiments of the groups II, III and IV showed a high value ($P<0.05$). The result for the prediction of the digestibility of the sample of group VI was similar to the in vivo determined digestibility.

The deviation in digestibility of one sample from group I was 11 percent units which resulted in differences in the ranking. Also in group V the ranking differed because of some values with considerable deviations. In these groups the correlation of the NIR results and in vivo measured digestibility were not very high.

It is unclear what the reason is for the large deviations of a few of the samples. There are however some general remarks which can be made. Firstly the wavelengths of the filters were chosen for single parameters like protein, fat and starch. These may be altered for future determination. Digestibility is a parameter which is affected by many factors and the optimal wavelength might not be present in the filterset chosen. Secondly, also the classical method has some sources of errors.

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COMPARATIVE RESULTS ABOUT AMINO ACID EFFICIENCY ESTIMATION BY DIFFERENT METHODS IN PIGS

F. Liebert, J. Tossenberger[1)], Z. Henics [1)] and G. Gebhardt

Division of Animal Nutrition Physiologie and Feed Science, Faculty of Agriculture, University of Leipzig, Germany

[1)]Division of Animal Nutrition and Feed Science, Faculty of Animal Production, Kaposvár, Hungary

Abstract

Comparative investigations with barley, wheat (2 charges) and soybean meal to determine "lysine quality" by different methods (N-balance trial, mobile nylon bag technique on faecal and ileal level, ileorectostomized pigs) have shown possibilities to use more time-sparing MNBT for lysine quality evaluation. More investigations are necessary for final conclusions. But all methods have to be proved in the area of practical application.

Introduction

In the field of a stepwise higher level to meet amino acid requirements for different performances in pigs more informations are necessary about the value of most important feeds to meet this requirements esp. for lysine, methionine/cystine, threonine and tryptophane.

Methodical developments in the last years show the following main directions in animal studies for this aim:

1. Absorption studies on praecaecal (or ileal) level with resp. without inclusion of endogenous losses (p.e. Varnish & Carpenter 1971; Taverner et al. 1981; Buraczewska et al. 1985)
2. Utilization studies following "slope ratio method" (Varnish & Carpenter 1975; Batterham 1985; Sato et al. 1987)
3. Utilization studies following physiological based model of N-utilization (p.e. Liebert & Gebhardt 1988)

Generelly these methods are time consuming, expensive, more or less useful for practical application and include different steps of utilization process (absorption resp. utilization). Beside intensive methodical clearing aspects of application become more and more interesting. New development in the field of time and costsparing in sacco methods could be successful if their results show significant correlations with accepted in vivo methods and can be verified in application experiments. First results of collaborative work will be discussed.

Materials and Methods

Based on the actual step of amino acid efficiency estimation for pigs (Liebert et al. 1990) we selected representative charges of important feeds in pig feeding to compare the results of amino acid quality evaluation by

A: N-balance trial, female pigs 30 - 50 kg live weight; model for N-utilization based on exponential function and in connection with utilization of limiting amino acid
B: Mobile nylon bag technique (MNBT) with in vivo predigestion and bag collection in faeces, 35 - 60 kg live weight (simple T-cannula in the duodenum)
C: Mobile nylon bag technique (MNBT) with in vivo predigestion and bag collection at the end of ileum, 35 - 60 kg live weight (simple T-cannula in the duodenum and PVTC-cannula (Van Leeuwen et al. 1988)

Predigestion for MNBT was realized with gastric cannulated pigs.
Prefeeding: conventional mixed feed (standard)
Time of incubation: 2,5 h
Number of bags per incubation: 10
Bag material: 25 x 40 mm, Nytex-mononylon, 50 micron
Bag content: 1 g

Immediately after incubation 2 bags were given into duodenal cannula at the same time (the following in a distance of 1 hour). After appearance in faeces (18...48 hours) resp. PVTC-cannula (3...5 hours) bags were carefully washed with destilled water and dried (60°C). We used 3 animals per feed (8 - 10 bags per animal). Bag contents of one animal were collected for N-analysis. The mixed sample from 3 animals served for amino acid analysis including Diaminopimelicacid (DAP). Based on DAP-content the quantity of bacterial nitrogen was calculated and used for correction of AA-absorption (AA content of bacterial protein was taken from Poppe & Meier 1983).

Results and Discussion

Results of our first investigations are summarized in table 1. Well known is the higher level of lysine absorption measured via faeces. More real dates for lysine evaluation, in comparison to balance method in vivo, were calculated for MNBT on ileal level, resp. on corrected ileal level. It's important to note a surprising agreement between N-balance trial and MNBT on ileal level without correction for bacterial lysine excretion in the case of barley and wheat 1. On the other hand there's no clear explantation for disagreement with wheat 2. Soybean meal also shows remarkable differences between evaluation methods.

Table 1. Comparative results about lysine evaluation in selected feeds for pigs.

	Barley ("Borwina 88")	Wheat 1 ("Miras 88")	Wheat 2 ("Zombor")	Soy-bean-meal, extr.
Lysine efficiency, %	89,0	84,0	94,0	83,0
Lysine absorption, %				
- MNBT (faeces) corrected	99,5	98,5	-	98,4
- MNBT (ileum)	89,0	83,2	82,1	91,5
- MNBT (ileum) corrected	92,5	90,5	88,8	94,4
- praecaecal (IRA)	-	70,2	-	-
Lysine content (g/16 g N)				
- brutto	3,06	2,32	2,55	6,80
- effective (N-balance)	2,72	1,95	2,40	5,64
- absorbable (MNBT ileum	2,72	1,93	2,09	6,22

A simple explanation therefore is the principal limitation of nylon bag technique in the field of real reflection of antinutritional factors in very small quantities of feed samples. From this point of view the results between N-balance trial and in sacco method in the case of soybean meal are not directly comparable. For wheat 1 we also estimated apparent lysine absorption with 3 ileorectostomized female pigs on ileal level. This result leeds to remarkable lower quality evaluation for lysine in wheat.

At least we need dates about effective resp. absorbable amino acid contents in feed proteins. Also in table 1 we calculated such values, based on estimation of lysine efficiency (N-balance trial) resp. lysine absorption (MNBT ileum). We found surprising agreement between these methods for barley and wheat 1, like discussed above. On the other hand we have found a range between 1,95...2,60 g/16 g N effective lysine in 16 different charges of wheat and also our calculation based on MNBT (ileum) is within this range. We left this range for wheat 1 by correction with apparent praecaecal lysine absorption of ileorectostomized pigs (1,63 g/16 g N).

Our results underline the essentiality of more comparative investigations in the field of amino acid quality evaluation for pigs and to examine these results in the area of practical application.

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SESSION 4

Methodologies for the measurement of digestion

METHODOLOGIES FOR THE MEASUREMENT OF DIGESTION

M.F.Fuller

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland

Abstract

Digestion is achieved by the actions of enzymes, some of which are secreted by the pig and others by the resident microflora. Since the utility to the animal of the end products is very different in the two cases, methodologies for the measurement of digestion require that these processes be distinguished. This is normally assumed to be achieved by measuring the flow of nutrients from the ileum. Techniques which have been developed for this purpose include various types of simple and reentrant cannulas and ileorectal anastomosis. Comparisons amongst these are presented, revealing, in some cases, important differences. The influence of microbial activity in the upper digestive tract on measured ileal digestibility is then discussed. Other approaches, the measurement of disappearance *in sacco*, of portal uptake and of utilisation by slope ratio assay are also described and some potential problems considered. Finally, the need to distinguish between apparent and true digestibility and some of the difficulties of doing so are discussed.

Introduction

There are few methodologies for the measurement of digestion alone; indeed, digestion by itself, the chemical breakdown of food constituents to simpler substances, is of less interest to nutritionists than the sum of the sequential processes of digestion and absorption. It is with these combined processes that this review will be concerned.

Before discussing available methodologies for the measurement of digestion and absorption it is important to examine the nature of these processes and consider the question of what information is required. Traditionally, 'digestibility' meant simply the proportion of any substance ingested which was not excreted in the faeces. Recognition that not only the total extent of breakdown and absorption but also the nature of the end products is important to the nutritive value of the food led to a need for a more detailed description of events in the gastrointestinal tract. The aim of this review is therefore to examine what is known of the nature and site of digestive activities within the gastrointestinal tract of the pig and assess the suitability of available methodologies for the measurement of digestion and absorption in the light of this understanding.

Components and sites of digestion

Digestion and absorption are both complex processes. Digestion is concerned with the breakdown of a multiplicity of food constituents but this review will concentrate on just two classes, proteins and carbohydrates. The breakdown of these results in the release of a wide range of simpler substances the absorption of which involves a number of different

transport systems.

The breakdown of food constituents occurs by both enzymatic and non-enzymatic cleavage but it is the the enzymatic processes which determine the ultimate extent of hydrolysis of any constituent. Enzymes in the digestive tract are produced both by the host animal itself and by its resident microflora. The ranges of activity include common elements but the microflora, acting singly or in concert, express important activities not possessed by the host and produce a different range of end products.

Extensive descriptions of protein and carbohydrate digestion by pigs have been published recently by Low & Zebrowska (1989) and Longland (1991).

Animal enzymes

The complement of enzymes secreted by the pig, in saliva, from the pancreas and from the mucosae of the stomach and small intestine include amylases, disaccharidases, lipase and phospholipases and a series of endo- and exo-peptidases with a range of amino acid specificities. Quantitatively the major end-products of these enzyme activities are the amino acids, small peptides, simple sugars, monoglycerides and fatty acids that are absorbed into portal blood and lymph and which are the primary nutrients for the animal.

Bacterial enzymes

Bacteria thrive in the intestine through successful competition with their host. In this they have two principle advantages. First, by lying **within** the lumen they have the possibility of utilising food constituents **before** they can be absorbed by the host. Second, they express a range of enzyme activities not possessed by higher animals and are thereby able to use food materials which would otherwise be voided unchanged. This applies particularly to plant materials with their variety of oligosaccharides and cell wall polysaccharides. The end products of the bacterial degradation of these materials are organic acids, lactate and volatile fatty acids, and microbial biomass. The nitrogen required for microbial growth is derived either from the diet or from the secretions of the host. The bacterial breakdown of cell walls not only provides the bacteria with carbohydrate and with the protein from the middle lamella but also releases from the cell a range of simpler carbohydrates and nitrogenous substances which, although susceptible to host enzymes, are probably rarely available to the host because of the prior intervention of the bacteria. Even so, the resistance of cell walls to degradation during transit of the non-ruminant digestive tract may be such that some escape entirely (Bach Knudsen & Eggum, 1984)

The end products of microbial digestion are inherently less useful to the host than the products of the host's own enzymic activity. Thus, proteins degraded in the large intestine contribute little, if at all, to the amino acid requirements of the pig (Zebrowska, 1973; 1975; Just *et al.*, 1981a). Similarly, although the colon is capable of transporting amino acids (Smith & James, 1976; James & Smith, 1976; Olszewski & Buraczewski, 1978), the infusion of lysine into the colon of a pig given a lysine-deficient diet did not significantly improve its rate of nitrogen retention (Wünsche *et al.*, 1984). Despite these demonstrations of the nutritional inutility of protein or amino acids reaching the large intestine there are suggestions to the contrary (e.g. Millward *et al.*, 1989). Such suggestions are based on labelling studies which show that infusion of ^{15}N-labelled ammonium salts results in the ^{15}N-labelling of amino acids in the body (Tanaka *et al.*, 1980). In most cases such labelling can be explained equally well by the uptake of ammonia, its incorporation into transferrable amino- groups which are then distributed by transamination to most other

amino acids. The expected exceptions are lysine and threonine, which have been shown not to transaminate. The finding that, following administration of ^{15}N-labelled ammonium salts, the label appeared in lysine and threonine (Deguchi & Namioka, 1989) suggests that this incorporation must indeed have been brought about by the gut microflora. However, even more surprising was the demonstration of labelling in these amino acids after giving ^{15}N-labelled ammonium salts to germ-free pigs (Deguchi *et al.*, 1978; Deguchi & Namioka, 1989). The physiological significance of these results is not clear; indeed, there is no obvious explanation as to how they could have arisen. Calculations based on the extent of labelling and the probable amino acid flux suggest that it could account for 3-5% of amino acid supply.

Distinguishing host and microbial digestion

Recognition that, especially for the proper measurement of amino acid digestibility but also for carbohydrate digestibility, digestion in the large intestine must be excluded, led to the development of methodologies to intercept digesta before they reach the caecum.

Several approaches have ben devised for separating these components of digestion. The earliest (Payne *et al.*, 1968), which is still used where it is more appropriate than any alternative, involved simply the slaughter of the animal and removal of digesta from a distal portion of the ileum. Because the transit of digesta along the small intestine is intermittent the terminal ileum may, at any one time, contain only small amounts of digesta and, especially with small animals such as rats and chicks, which are often used for such assays, it may be necessary to pool digesta from several animals to have enough for analysis. Since this not allow any assessment of variation between animals it is important to replicate the group observations.

At death, there is rapid shedding of mucosal cells into the gut lumen (Badawy *et al.*, 1957; 1958; Fell, 1961) which would create an artefact in the determination of the composition of ileal digesta; to avoid this possibility the digesta must be sampled whilst the animal is under terminal anaesthesia.

The need to slaughter animals makes this approach expensive when applied to pigs and other large species. Furthermore, the fact that only one observation can be made with any one animal means that individual animal variation cannot be taken into account in the statistical analysis of results, for which replicated observations in the same animal would be greatly preferable. For these reasons various techniques have been developed to allow digesta to be obtained repeatedly from the same animal.

The simplest technique for obtaining digesta continuously is by the use of a T-cannula permanently implanted in the ileum (Cunningham *et al.*, 1963). Such cannulas are well tolerated for long periods, generally limited only by the animal's outgrowing them. Simple T-cannulas rely on natural forces to divide the flow, one part continuing along the intestine, the other diverted through the cannula. The assumption is made that this provides a perfectly representative sample of the total flow; that the division of the flow does not involve any fractionation of components. This assumption is not often rigorously examined and may be an important source of error, especially with high-fibre diets. It is a matter of common observation that the stem of a cannula can become blocked with fibrous material that is clearly **not** representative of the total flow. A further assumption is that the marker flows in constant proportion to the nutrient of interest and this assumption, too, is rarely tested. The use of markers will not be reviewed here (see e.g. Uden *et al.*, 1980) but, in any event, it is important that digesta are collected for a sufficient period to be representative of the daily average flow.

The use of markers is obviated by collection of the entire ileal digesta flow which can be achieved by the use of re-entrant cannulas (Easter & Tanksley, 1973). The conventional re-entrant cannula has two disadvantages. First, it requires complete transection of the small intestine, interrupting the transmission of the normal migrating myoelectric complex which is necessary for normal digesta passage. Second, the dead space of the cannula, when connected in the re-entrant mode, is such that blockage is a common occurrence with all except finely ground diets. This precludes the examination of many normal feed ingredients without regrinding, which can be self-defeating.

These problems were overcome to some extent by the introduction of the technique of ileo-caecal re-entrant cannulation introduced by Darcy *et al.* (1980). In this approach the caecum is resected to accomodate the proximal (sampling) cannula which is thus situated immediately distal to the ileo-caecal junction. Although, in this technique, the integrity of the small intestine is maintained, the surgery is inherently more traumatic than with a simple T-cannula, with ablation of the caecum, the re-entrant cannula being placed in the proximal colon. Unlike a conventional re-entrant cannula, there is no connection between the two parts, digesta being collected continuously from the proximal cannula and returned intermittently through the distal one. Even with this technique, however, there have been problems with coarsely ground or fibrous diets which cause blockage of the proximal cannula.

In an attempt to avoid such problems we have experimented with a one-piece post-valvular ileo-caecal re-entrant cannula (A.P. Drake & M.F. Fuller, unpublished). The cannula was designed on the same principle as that described by Ivan (1974) but of larger diameter, approximately 3cm, and with an overall length of 9cm. After the pigs had recovered from surgery the cannulas were evaluated by giving diets with added chromic oxide to some pigs, returning unmarked digesta from other pigs to the caecum. We failed to recover all the dietary chromium in the digesta; on the contrary, chromium was found in the faeces showing that some digesta had bypassed the cannula. At post mortem examination it was evident that a sac had formed round the cannula allowing digesta to move from the ileum into the colon without passing through the cannula. It seems that only by providing separate fistulas through the body wall for the ileal and caeco-colic cannulas can these adaptations of the gut tissue be avoided.

Van Leeuwen *et al.* (1988) and den Hartog *et al.* (1988) have proposed a solution to the problems of re-entrant cannulas by the use of a 'postvalvular T-caecum cannula', using a simple cannula in the caecum which can be opposed to the ileo-caecal valve allowing digesta to be collected as they leave the ileum.

To avoid the use of any cannula but yet to be able to take numerous samples from the same animal, Livingstone and I (1982) explored the use of ileo-rectal anastomosis. Our first animals had a simple end-to-side anastomosis of the terminal ileum to the rectum, allowing residual contents of the colon to be evacuated along with digesta from the ileum. On *post mortem* examination after some weeks it was evident that, although there was extensive atrophy of the large intestine there was reflux of ileal digesta into the distal colon. Picard *et al.* (1984) modified our technique by isolating the large intestine completely but providing a cannula in the distal colon for the evacuation of residual digesta and gases. We adopted this modification in our later work as did Green *et al.* (1987a) and Laplace *et al.* (1989).

Ablation of the caeca in poultry has long been used similarly to minimise the fermentative component of digestion in these species (Payne *et al.*, 1971).

Comparisons of methodologies.

Since the development of several alternative methodologies for the measurement of precaecal digestion many measurements of ileal digestibility have been made and tables of digestible amino acids, for example, compiled (e.g., Heartland Lysine, 1988). Although these tables are an important tool for practical diet formulation it is important to enquire whether results obtained by the different methods are compatible. The first question perhaps concerns the normality of cannulated animals.

Livingstone & McWilliam (1985) found that pigs with simple cannulas had a similar voluntary food intake to unmodified controls but grew approximately 7% slower, suggesting that the presence of a simple cannula had only some slight effect on nutritional performance. Jørgensen *et al.* (1985) reported that cannulated pigs, whilst growing at a similar rate to intact animals, digested the dry matter, nitrogen and lysine in most diets to a greater extent than controls. Moughan & Smith (1987) compared slaughter and T-cannulas and found no difference. In comparisons between simple and re-entrant cannulation Zebrowska *et al.* (1977) found some significant differences but with no consistent pattern. Taverner *et al.* (1983) compared simple and re-entrant cannulas with three diets and found no significant differences in the digestibilities of dry matter, nitrogen or amino acids (Table 1).

Darcy-Vrillon & Laplace (1985) compared ileorectal anastomosis and ileocolic postvalvular re-entrant cannulation (ICPV). Their results are shown in Table 2.

Table 1. Comparison of estimates made with simple or re-entrant cannulas of the digestibility of dry matter, nitrogen and amino acids in wheat, lupin and meat-and-bone meals by pigs. Results of Taverner *et al* (1983).

cannula	re-entrant	simple	s.d.
Dry matter	0.706	0.688	0.0210
Nitrogen	0.794	0.800	0.0204
Amino acids (average)			
apparent	0.809	0.816	0.0517
true	0.855	0.862	0.0475

Table 2. Comparison of post-valvular ileo-colic re-entrant cannulas and ileo-rectal anastomosis in estimating the digestibility of dry matter, nitrogen and the sum of 17 amino acids (ΣAA) in a standard compound diet (C) and in diets with 45% wheat bran (WB) or 32% sugar beet pulp (SBP) added to a purified diet. Differences marked * were significant ($p<0.05$). Data of Darcy-Vrillon & Laplace (1985).

Diet	C		WB		SBP	
Technique	IRA	ICPV	IRA	ICPV	IRA	ICPV
Dry matter	0.60	0.63	0.62	0.76*	0.68	0.81*
Nitrogen	0.70	0.67	0.78	0.82	0.74	0.87*
Σ AA	0.76	0.75	0.84	0.88	0.81	0.90*

In a comparison of several methods invoving pigs, rats and chickens (M.F.Fuller, B.Darcy-Vrillon, J.-P.Laplace, M.Picard, A.Cadenhead, M.Jung, D.Brown & M.F.Franklin in preparation) three isonitrogenous diets were used, one with barley as the sole protein source, one with milk and one with an isonitrogenous mixture of the two. The methods compared were: 1 (IRA1) ileorectal anastomoses with pigs (Laplace *et al.*, 1989) 2-5 weeks after surgery; 2 (IRA2) the same pigs when they weighed 80kg; 3 (T-C) T-cannulas with pigs of ca. 100kg; 4 (C-) caecectomised cockerels (Green *et al.*, 1987b; McNab & Fisher, 1981); 5 (ICPV) postvalvular ileo-colic cannulation (Darcy *et al.*, 1980); 6 (IRA3) ileorectal anastomoses with pigs (Laplace *et al.*, 1989) 2-5 weeks after surgery; 7 (R-) *ileorectal anastomoses with* rats (M. Picard, unpublished). The work was done at three centres, the Rowett Institute (methods 1-3), Centre Nationale de Recheches Zootechniques, Jouy-en-Josas (methods 5 & 6) and Alimentation Equilibrée Commentry (methods 4 and 7).

There were considerable differences between results with different techniques and with different species, but the differences between results obtained in different trials using the same technique were equally great, suggesting that the variation between trials is of as much concern as the variation between methodologies or species. A summary of these results is given in Table 3.

Table 3. Apparent and true digestibilities, averaged over nitrogen and all amino acids, in seven trials with three diets, based on barley (B), dried skimmed milk (M) and an isonitrogenous mixture of the two (BM). For details of the methods compared see the text above. Data of M.F.Fuller, B.Darcy-Vrillon, P.-P.Laplace, M.Picard, A.Cadenhead, M.Jung, D.Brown & M.F.Franklin, in preparation

	Digestibility							
	Apparent				True			
diet	B	BM	M	mean	B	BM	M	mean
trial								
IRA1	0.58	0.66	0.70	0.65	0.70	0.71	0.78	0.73
IRA2	0.60	0.61	0.80	0.67	0.65	0.66	0.83	0.71
T-C	0.59	0.81	0.76	0.72	0.70	0.92	0.89	0.84
C-	0.63	0.70	0.64	0.66	0.82	0.88	0.87	0.86
ICPV	0.76	0.87	0.85	0.83	0.84	0.95	0.96	0.92
IRA3	0.66	0.75	0.82	0.74	0.76	0.84	0.94	0.84
R-	0.69	0.75	0.81	0.75	0.78	0.82	0.90	0.83
mean	0.64	0.73	0.77	0.72	0.75	0.83	0.88	0.82
sed		0.019		0.064		0.018		0.073

Table 4 Digestibility of dry matter and nitrogen in a standard cereal-based diet by pigs with simple T-cannulas (T-C) and pigs with ileo-rectal anastomoses (IRA). Each value is the mean for 6 animals. Data of M.F.Fuller, R.M.Livingstone & B.F.Fell (unpublished).

Months after surgery.	organic matter		nitrogen	
	T-C	IRA	T-C	IRA
3	0.67	0.68	0.71	0.69
6	0.68	0.72	0.72	0.72
12	0.72	0.73	0.75	0.74
18	0.72	0.73	0.75	0.72
24	0.70	0.70	0.72	0.73
mean	0.69	0.72	0.73	0.73
s.e.d.	0.009 ($p<0.01$)		0.009 (n.s.)	

Long term effects of ileorectal anastomosis.

With my colleagues Livingstone and Fell, I maintained pigs with ileo-rectal anastomosis for up to 2 years after surgery and we compared their digestion with that of pigs with T-cannulas. The results are shown in Table 4.

The functional destruction of the large intestine is not without effect on the pig. The first and most obvious consequence is the much greater loss of water and electrolytes and it is necessary to provide large supplements of sodium and other mineral elements to compensate for these losses. At first after surgery ileal digesta pass frequently from the anus and at this stage are similar in composition to those obtained from cannulated pigs but after some time their frequency decreases as does their water content. These changes suggest a progressive modification of the function of the terminal ileum. Morhological and histological examination of the gut 26 weeks after surgery showed histological changes in the ileum with increased goblet cell numbers, hypertrophy of smooth muscle, elongation of the crypts and and atrophy of the enterocytes at the villus tips. Concentrations of volatile fatty acids in the digesta voided by these animals were also higher than in digesta from pigs with T-cannulas. These changes suggest that the ileum had adapted to assume some of the functions of the bypassed large intestine. This adaptation had evidently affected to some extent the digestion of organic matter, especially in the first few months, but not that of nitrogen.

In view of the doubts to which this evidence gave rise concerning the functional state of the ileum, as well as from ethical considerations (it is extremely difficult to avoid the pig suffering discomfort from the frequent outpouring of digesta from the anus) we discontinued the use of ileo-rectal anastomosis as a means of obtaining ileal digesta.

Comparability of pigs and poultry

The possibility of using data obtained in the more rapid assay which is possible with poultry to obtain data which may be used to predict digestibility values for pigs led Jackson (1990) to make the comparison with some 30 feed ingredients. The results are shown in Table 5.

Table 5. Comparison of true amino acid digestibilities determined with poultry and apparent amino acid digestibilities determined with pigs (T-Cannulas) in 30 raw materials. Data of Jackson (1990). * Significant differences ($p<0.05$).

Amino acid	Poultry	Pigs	Difference between species
arginine	0.83	0.82	0.01
histidine	0.83	0.79	0.04
isoleucine	0.82	0.76	0.06*
leucine	0.87	0.82	0.05
lysine	0.70	0.72	-0.02
methionine	0.85	0.78	0.07*
cystine	0.76	0.73	0.03
phenylalanine	0.86	0.81	0.05
threonine	0.77	0.70	0.07*
valine	0.81	0.78	0.03
alanine	0.82	0.78	0.04
average	0.81	0.77	0.04

The regression relating the pig measurements (D_p) to those obtained with the caecectomised cockerels (D_c) was

$D_p = 0.26 + 0.63\ D_c\ \ r^2\ 0.54$

Measurements *in sacco*

With conventional measurements of ileal digestibility only one material can be tested at a time. The technique of measuring the degradation of many materials simultaneously in the rumen by the use of dacron bags led to the development of similar *in sacco* techniques in pigs (Sauer *et al.*, 1983; Livingstone, 1985; Graham *et al.*, 1985). Since the bags used to hold the feed material cannot pass normally through the pylorus it is neccesary to provide an acid/pepsin predigestion *in vitro* and to introduce the bags into the small intestine through a cannula in the duodenum. Thereafter, the bags can be retrieved from the faeces in order to estimate overall digestion; to estimate precaecal digestion requires that the bags be intercepted at the end of the ileum, reintroducing the technical problems already outlined of blockage and modification of rate of passage. The choice of a suitable pore size is also problematic, requiring a compromise between the free mixing of the test material with intestinal secretions that is allowed by large pores and preventing the escape of small particles of indigestible material released by the breakdown of surrounding or associated structures. This is probably a major factor responsible for disagreement between *in sacco* and *in vivo* estimates (Graham *et al.*, 1985; Metz *et al.*, 1985; Taverner & Campbell, 1985)

A further question in the use of *in sacco* methods concerns the isolation of the material from contact with the gut wall. There are two grounds for concern. The first is that material confined within the bag is never able to come into contact with membrane-bound hydrolases; the second is that certain feed constituents which in normal digestion would

react with the gut wall are prevented from doing so (Pusztai *et al.*, 1991).

Measurement of uptake

An alternative approach, which is especially suitable for studying the kinetics of digestion and absorption, involves the measurement not of the **disappearance** of constituents from the digestive tract but the uptake into portal blood of the end products of their degradation. This requires measurements of arteriovenous differences (systemic arterial blood to portal venous blood) in concentration to be multiplied by the rate of blood flow in the portal vein (Rérat *et al.*,1980). It is important to appreciate that such measurements of uptake will differ from measurements of disappearance because of the metabolic activity of the gut wall (Souffrant *et al.*, 1986)

Significance of microbial activity in the upper gastrointestinal tract

Although microbial activity is principally associated with the large intestine, it is by no means confined to that part of the gastrointestinal tract. One factor which tends to minimise the impact of the microflora of the upper digestive tract is that the residence time of digesta in the small intestine is much shorter than in the caecum and colon limiting the time available for fermentation. Nevertheless, in recent years there has been increasing evidence of substantial degradation of non-starch polysaccharides in the small intestine (Millard & Chesson, 1984; Graham *et al.*, 1986; Buraczewska *et al.*, 1988; Longland *et al.*, 1988). Since there is no evidence of any mammalian enzyme capable of catalysing these hydrolyses this must be taken as evidence of a commensurate degree of microbial activity.

Depending on the physiological state of the animal and on the nature of its food microbial activity in the upper digestive tract can therefore be substantial and of considerable nutritional significance (Dierick et al., 1986; Jensen et al., 1987; Jensen,1988). This is illustrated by the results of Jensen *et al.* (1987; Table 6).

Although ATP concentration is highest in the caecum and proximal colon, values in the last portion of the small intestine are one third to one half as high as in the caecum; furthermore, due to the ready availablity of easily fermented substrate there, the metabolic activity of these organisms, as evidenced by their adenylate energy charge (Jensen *et al.*, 1987) is higher than in the caecum. There is no doubt, therefore, that the measurement of digestibility at the terminal ileum is not a measure of host digestion alone but includes a good deal of microbial activity. The question arises as to the extent to which the end products of this microbial metabolism differ from those of the host digestion which would have taken place in its absence.

One means of eliminating, or at least reducing, the effects of the enteric microflora is through treatment with antibiotics. Although even large doses of antibiotics can only reduce and never completely eliminate gut bacteria such experiments show clearly that digestibility up to the end of the ileum is increased and that in the large intestine is reduced by antibiotic treatment (Just *et al.*,1981b, 1985; Drake, 1990). Even if, by this means, the enteric population can be reduced by only one order of magnitude 90% of the bacteria have been removed and, other things being equal, one might expect that there would be only one tenth as much activity as in untreated animals, allowing extrapolation to the unattainable condition of a sterile gut. A sterile gut can, of course, be achieved by

Table 6. ATP concentrations (μg ATP/g dry matter) in various parts of the digestive tract of pigs given diets based on wheat flour or wheat flour + wheat bran. Data of Jensen *et al.* (1987).

	Diet	Flour	Bran
Segment			
stomach	1	13	31
	2	7	27
small intestine	1	13	45
	2	48	80
	3	166	108
caecum		324	240
large intestine	1	84	228
	2	51	168
	3	19	90
	4	9	90
	5	7	61
	6	6	36

raising animals from birth in germ-free conditions, but the substantial differences in the morphology and histology of the gut between germ-free and conventionally florated animals raise doubts about the relevance of such observations. By extension, the same reservations must apply to the use of antibiotics though in this case the gut has been normally colonised from birth until the beginning of treatment and it is unlikely that the effects of the microbes on gut development are reversed in the few days of antibiotic treatment.

In vitro assessment of digestion.

There are several motives for the development of *in vitro* systems. These are reviewed elsewhere in this volume but it is worth pointing out in the present context that, by their nature, such assays can avoid the problems of microbial intervention in the assessment of digestion. They cannot, however, take account of those factors peculiar to the animal such as its endogenous secretions or its reactions to antinutritional factors.

Amino acid availability

Chemical alterations to the amino acids in a food protein may be brought about by processing with heat or strong chemicals. Such compounds, though they may be released from the protein during digestion and though they may be absorbed, are nevertheless of reduced nutritional value, if indeed they have any value at all. It would therefore seem important to determine not only the division of total amino acid flow through the gut between that which is absorbed and that which is not but also the division of the available

and unavailable fractions.

Integrative assays

The eventual aim of any methodology for evaluating a food is to estimate its contribution to the nutrient requirements of the animal. Thus, assays in which foods limiting in a particular nutrient are compared with that nutrient given in purified form might be expected, in theory at least, to integrate the combined effects of digestibility, availability and antinutritional factors in a single measure which expresses the effective nutritive value of the food. For example, in the case of amino acids, slope ratio assays have been used to compare the growth response to increments of amino acid in the food to that obtained to increments of the crystalline amino acid when both are added to a basal diet specifically limiting in that one amino acid alone (e.g. Leibholz *et al.*, 1986). As shown by Batterham *et al.* (1990) differences in ileal digestibility of amino acids do not necessarily correspond to differences in availability assessed by slope ratio assay. An inevitable feature of this approach is that whilst increments of the free amino acid **improve** the quality of the dietary protein increments of the test food do so to a lesser extent, if at all. As a result, there may be an exaggerated response to the free amino acid resulting from the rectification of an amino acid imbalance (Sato *et al.*, 1987).

Apparent and true digestibility

The distinction between apparent and true digestibility is an important part of the methodology of measuring digestion since, to allow full additivity of estimates on individual ingredients in the formulation of complete diets, it is necessary to work with true digestibilities, accounting separately for the endogenous losses from the gut which will be in part a function of the animal and in part of the diet. Endogenous losses during digestion form the subject of a separate contribution to this symposium (Souffrant *et al.*, 1991) and will not be discussed in detail here; nevetheless, there are some points which are relevant to the choice of methodologies.

The true digestibility of proteins is difficult to determine directly. The survival through the gastrointestinal tract of pure proteins may be detected immunologically in digesta (e.g., Pusztai *et al.*, 1990) but even simple feed ingredients normally contain a large number of distinct proteins.

The contribution of endogenous secretions to the flow of a nutrient out of the ileum or out of the rectum is conventionally determined by feeding a protein-free diet. This method has been criticised as altering the protein status of the animal and thereby, perhaps, altering the rate at which proteins are secreted into the gut lumen. An alternative often advocated is to feed a series of diets of different protein content and extrapolate to zero protein intake. It is worth pointing out that this approach rests upon exactly the same assumption, that feeding protein does not alter the rate of endogenous secretion, all the increase in nutrient flow being interpreted as reflecting the inherent digestibility of the material when it may very well derive from a combination of the two sources.

Labelling methods, whether using labelled dietary proteins (e.g. Partridge *et al.*, 1985) or labelling the animal and its secretions (e.g., Krawielitzki *et al.*, 1977; de Lange *et al.*, 1989) may help to elucidate the contribution of endogenous constituents to ileal nitrogen flow. However, the uptake of amino acids and their incorporation into secreted proteins by the intestinal epithelial cells can be very rapid; further, it appears that the precursor amino acids for protein synthesis by the enterocytes are derived both from the lumen and the

blood (Alpers, 1972; 1983). Thus, the recycling of luminal amino acids can lead to an underestimate of endogenous secretions.

The endogenous secretions entering the intestinal lumen originate from many sources and include a wide variety of substances. During their passage through the gut many of these substances are themselves subjected to enzymic attack, allowing their constituents to be recovered. This is especially important with the amino acids which can be recovered from enzyme secretions and from desquamated enterocytes. From the point of view of estimating the true digestibility of dietary constituents, therefore, the identification of all the materials secreted into the gut is of less importance than identifying and quantifying those which are not recycled. Because of their resistance to degradation the mucins are likely to account for a substantial fraction of these losses, with major contributions to the losses of serine, proline, threonine and cystine. It was for that reason that we have recently examined the flow of galactosamine from the ileum with a view to estimating the contribution of mucins to ileal nitrogen outflow and the constancy of the ratio of galactosamine to total nitrogen in that outflow (Fuller & Cadenhead, 1991).

A further substantial but variable component of endogenous ileal outflow is contributed by bacteria. As discussed above, the extent to which non-starch polysaccharides are degraded before they reach the caecum predicates a vigorous microbial metabolism. This results not simply in the utilisation of dietary amino acids for bacterial protein synthesis but also in the degradation of some amino acids and the synthesis of others. The contribution of microbial protein to the total flow of amino acids leaving the ileum seems therefore to deserve further study if we are to develop methodologies which allow us to estimate acurately the net uptake of amino acids from the digestive tract.

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CHRONIC MODELS FOR THE EVALUATION OF THE GI TRACT FUNCTION DURING PORCINE POSTNATAL DEVELOPMENT

S.G.Pierzynowski, B.R.Weström and B.W.Karlsson

Department of Animal Physiology, University of Lund, Helgonavägen 3B, S-223 62 Lund, Sweden

Abstract

A technique for sampling pure unactivated pancreatic juice from young developing pigs up to 10 weeks of age was developed. A pancreatic duct catheter, a re-enterant duodenal fistula for permitting periodic external juice sampling and a jugular catheter for blood sampling were surgically implanted in each pig when they were 1 - 10 weeks old. The procedures did not appear to interfere with normal growth or with the physiological responses to feeding and hormonal stimulation with CCK and secretin. A model for the in situ perfusion of the pancreas was also developed where, in addition to the above described catheterizations, chronic catheters were inserted into the superior pancreatic artery and the portal vein. Using this model, the effect of the direct infusion of pancreastatin on the exocrine end endocrine pancreas was explored.

To investigate insulin tolerance and tissue capacity for glucose utilisation, a method using an extracorporeal blood circulation system via two jugular catheters connected to a feedback system for blood glucose measurement and glucose/insulin infusion was developed.

For investigation of liver function, catheters were inserted into the jugular, portal and hepatic veins, the latter directly via the visceral surface of the left liver lobe, and in the bile duct. With this model the intestinal absorption, liver metabolism, and biliary excretion of the vasopressin analogue dDAVP was determined after intraduodenal, intraportal and intrajugular routes of administration.

Keywords: Chronic pig models, GI tract, pancreas, liver, weaning, postnatal development.

Introduction

Physiological functions of the GI tract in adult pigs have been studied in chronic experiments, especially with respect to pancreas (Wass 1965, Corring et al. 1972, Zebrowska et al. 1983) and liver functions (Juste et al. 1983, Yen and Killefer 1986). However, during immediate postnatal development and around the time for weaning, both periods characterised by rapid and extensive changes in GI tract functions, these aspects have been studied generally after slaughter of the animals, i.e., the exocrine pancreas has been studied using homogenates (Corring et al. 1978, Owsley et al. 1986, Weström et al.1987) or acute experiments (Harada et al. 1989).

However, chronic experiments are to be preferred, since studies on homogenates only provides static information at a given time-point, and acute experiments may be influenced by the anaesthesia (Cuber et al. 1989). Moreover, the lack of regulatory pathways in in vitro perfused organs limits the use of these models for the evaluation of normal functions. Chronic models, especially during postnatal development, can relevantly describe the dynamic functions of organs, eg. the real secretory capacity, even if they are time consuming and difficult to perform.

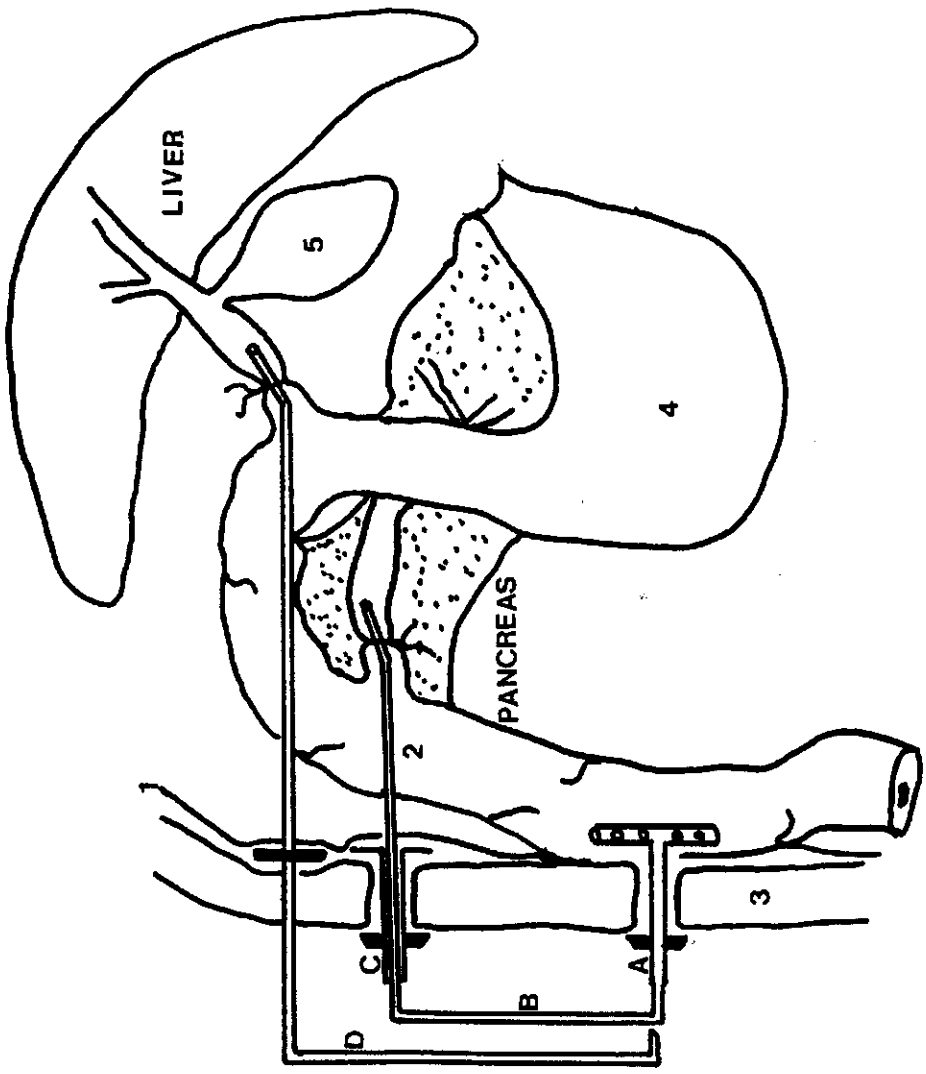

Fig.1. Schematic drawing of the operation site for pancreatic and bile duct catheterisation:
A - perforated T-cannula, B - pancreatic catheter, C - abdominal cannula, D - bile catheter.
1 - peritoneum, 2 - duodenum, 3 - abdominal wall, 4 - stomach, 5 - gall bladder.

In the present paper we describe four surgical models based on a series of chronic experiments. These models are suitable for the study of the pancreas and liver functions during postnatal development from 1st week of age to after weaning in the piglet.

Description of the chronical experimental model

1. Catheterisation of the exocrine pancreas for the external collection of pure pancreatic juice.

The pancreatic duct in piglets was catheterised from the 1st week of life according to Pierzynowski et al. (1988, 1990) and as shown schematically in Figure 1. A pancreatic duct catheter (i.d. 0.3 mm, e.d. 0.64 mm) was exteriorised via a "protection", an abdominal cannula which was placed between the peritonium and muscle layers in the first right intercostal space. The duct catheter was usually replaced around the 3rd week of life by a larger one (i.d. 0.64 mm, e.d. 1.19 mm) with a silicon ring placed between the muscle layers and peritonium after the abdominal cannula had been removed. The large type of catheter was also routinely used directly for duct catheterisation from 3rd week of life. Between the experiments, the duct catheter was connected to a perforated T-cannula placed in the jejunum, thus maintaining a constant re-enterant flow of juice. For hormone infusion and blood sampling a catheter was also implanted in the jugular vein (Fig.2). The unweaned operated pigs were returned to their litters; after weaning at 4-6 weeks, they were housed in separate pens having visual contact.

2. Pancreas perfusion in situ and catheterisation of the pancreas duct

After pancreatic catheter and jejunal fistulae implantations as described above, a perfusion catheter was inserted with the tip in the superior pancreatic artery through the lienal artery as schematically shown in Figure 2 (Ahre'n et al. 1990). A second catheter for blood sampling was inserted in the portal vein with its tip in the liver hilus via the lienal vein.

3. Extracorporal blood circulation connected to a feedback system for glucose measurement and glucose/insulin infusion

An extracorporal blood circulation system for blood sampling and infusions has been developed in pigs ca 6 weeks old (Pierzynowski et al. 1990). Two silicon catheters were inserted into the jugular vein (6 cm for the withdrawal catheter and 8 cm for the infusion catheter) through a longitudinal nonpenetrating purse string suture. The catheters, exteriorised under the skin to the dorsal part of the neck, were connected via the ex vivo circuit to a portable Insulin Glucose Closed Loop Regulatory System (Gambro, Lund, Sweden) for glucose level measurements and glucose/insulin infusion. By this ex vivo circuit, blood from the jugular vein was pumped at a flow of 10 ml/min. through a silicon tube (i.d. 3 mm) and back. The blood glucose monitor was inserted into the ex vivo circuit at the point of the withdrawal catheter. From 3 consecutive measurements the proper glucose infusion rate was calculated, and then together with insulin automatically infused via the infusion catheter to obtain a steady blood glucose level.

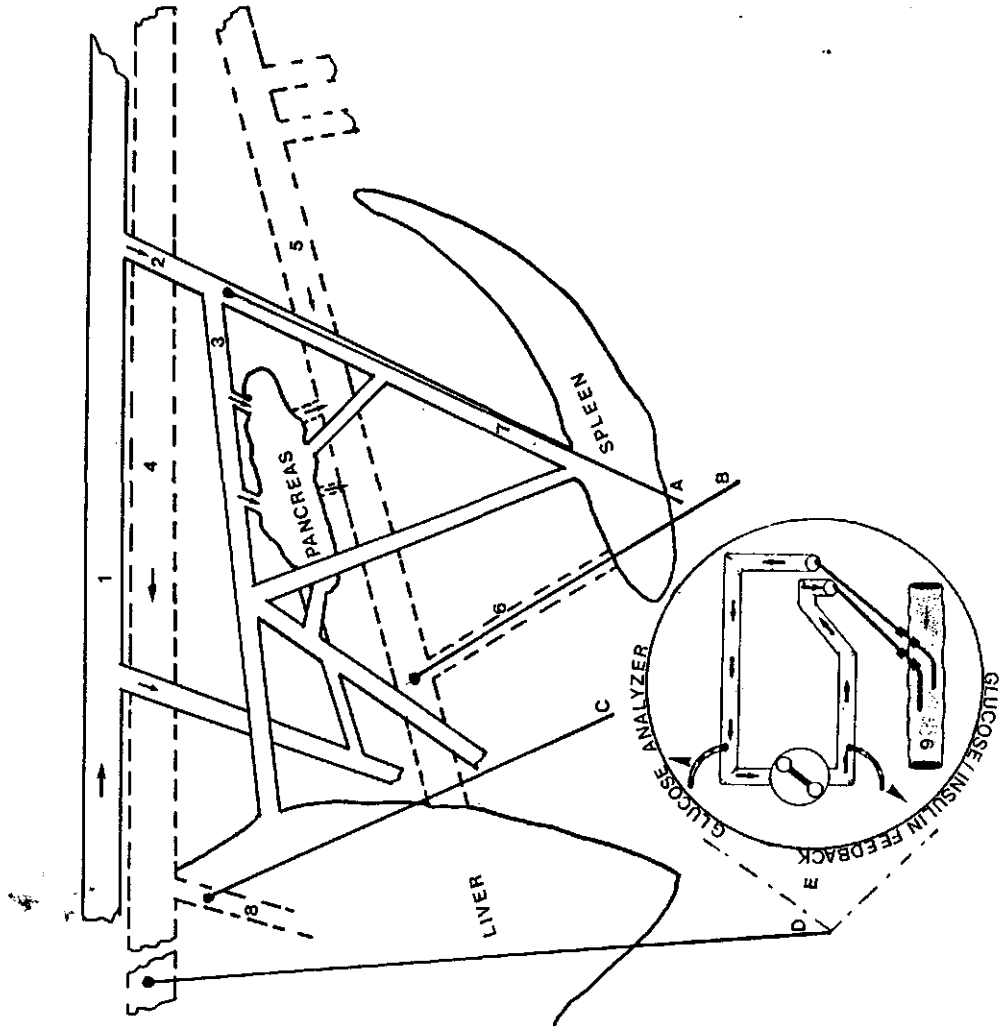

Fig.2. Schematic drawing of the operation site for visceral vessels and jugular vein catheterisation:
A - pancreatic arterial catheter, B - portal catheter, C - hepatic catheter, D - jugular catheter, E - extracorporal circulation units.
1 - abdominal artery, 2 - celiac artery, 3 - superior pancreatic artery, 4 - main posterior vein, 5 - portal vein, 6 - spleenic vein, 7 - spleenic artery, 8 - hepatic vein, 9 - jugular vein.

4. Liver perfusion and catheterisation of the bile duct

In this model infusion/blood sampling catheters were inserted in weaned pigs (8-10 weeks old) into the portal and hepatic veins (Fig. 2) from an incision in the first left intercostal space (Lundin et al. 1990). The portal catheter was inserted via the lienal vein, the hepatic catheter directly via the visceral surface of the left liver lobe. A peripheral blood sampling catheter was also implanted in the jugular vein (Fig.1,2). For the periodic collection of bile, a catheter was implanted in the bile duct together with a re-enterant jejunal T-cannula from an incision in the first right intercostal space.

Applications of the methods

Nursing piglets were fitted with a chronic pancreatic catheter to sample pure pancreatic juice during porcine postnatal development up to and after weaning both during basal and postprandial conditions and after hormonal stimulation with CCK and secretin (Pierzynowski et al. 1990). The results showed that during the sucking period, the exocrine functions remained low, whereas after weaning, pronounced increases of juice outflow and output of enzymes took place, both basally and post prandially. Furthermore, the response to CCK and secretin stimulation increased with age after the first two weeks of life.

In a study utilising the more complex pancreas model, pancreastatin was infused in vivo into the pancreas circulation via the artery catheter, for the first time into its species of orgin (Ahre'n et al. 1990). It was found that pancreastatin had an inhibitory effect on both the exocrine pancreas and insulin secretion.

To study the difference in glucose consumption between healthy pigs and pigs with alloxan/streptozotocin induced diabetes (6 weeks old, weaned) the model with extracorporal blood circulation coupled to the glucose monitor /closed loop feedback control system was used on conscious animals (Pierzynowski et al. 1990). It was found that glucose consumption and the glucose/insulin ratio were higher during the infusion of insulin in the healthy pigs than in the diabetic ones.

Intestinal absorption, liver metabolism and bilary excretion of the vasopressin analogue dDAVP were determined following intrajugular, intraportal and intraduodenal routes of administration, in pigs surgically prepared according to the last model. It was found that the intestinal mucosa constitutes the major barrier to the absorption of dDAVP. Furthermore, the results indicate that dDAVP is degraded in the liver and that it is excreted into the bile in small amounts.

Perspectives

Many of the physiological functions of the GI tract remain to be understood, especially those occurring during postnatal development which might influence general health and productivity, through to the adult animal. To determinine the types of food which can be easily digested, absorbed and utilised in early postnatal life and around weaning are important goals. The chronic models presented here for studying pancreas and liver functions will contribute to solving some of the basic problems with respect to the gastrointestinal development of the pig. These pig models, are not only useful for studying pig development, but are also convenient for studying developmental aspects of the GI tract in general, especially as models for human development (Miller and Ullrey 1987).

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COMPARISON OF VARIOUS TECHNIQUES FOR MEASURING ILEAL DIGESTIBILITY IN PIGS

Torsten Köhler[ab], Martin W.A. Verstegen[b], Joop Huisman[c], Piet van Leeuwen[c], and Rainer Mosenthin[a]

[a] Institute of Animal Nutrition and Feed Science, University of Kiel, D-2300 Kiel, FRG.

[b] Department of Animal Nutrition, Agricultural University, Wageningen, The Netherlands

[c] TNO Institute for Animal Nutrition and Physiology (ILOB), Wageningen, The Netherlands

Abstract

The objective of this study was to compare different techniques for collection of ileal digesta in pigs. In experiment 1 pigs were either fitted with the recently developed **Post-Valve T-Caecum (PVTC)** cannulas, or **re-entrant cannulas** or **simple T-cannulas** to compare ileal nutrient digestibilities obtained by these different methods. In experiment 2 ileal nutrient digestibilities of three different feedstuffs were measured in pigs fitted with PVTC cannulas. These results were compared with corresponding results from the literature in which re-entrant cannulas, or simple T-cannulas or ileorectal anastomosis were used. Experiment 3 included comparative measurements of ileal nutrient digestibilities in pigs fitted with PVTC cannulas or surgically modified by the **ileo-rectal anastomosis technique (IRA)**. There were no differences between the different cannulation techniques in ileal digestibilities of the parameters measured, except for digestibility of nitrogen (N) and Neutral Detergent Fibre (NDF) measured in PVTC and re-entrant cannulated pigs, respectively. However, ileal digestibilities of dry matter and Na were higher in IRA pigs than in PVTC pigs whereas ileal digestibilities for N(except in period 3), amino acids (except threonine in period 2 and 3) and K were lower ($P < .05$ - $P < .001$). Digestibilities of crude protein and lysine of three different feedstuffs and measured in PVTC cannulated pigs were comparable with results from the literature which were obtained with different digesta collection techniques. It can be concluded that PVTC cannulation is an appropiate alternative for digestibility measurements at the distal ileum of pigs.

Introduction

Digestibility measurements at the terminal ileum of pigs have been accepted to be more appropiate than faecal measurement (Zebrowska, 1973). Different techniques have been developed during the last two decades to collect ileal digesta. In general four different methods are in use: 1. the cannulation of the ileum anterior to the ileo-caecal valve (simple T-cannula, ileo-caecal re-entrant cannula), 2. the cannulation of the intestinal tract post-valvular (the ileocolic post-valvular procedure as described by Darcy et al. (1980), 3. the Post-Valve T-Caecum cannula from van Leeuwen et al. (1991)) and 4. the ileo-rectal anastomosis (a bypass technique of the colon) as described by Picard (1984), Darcy-Vrillon & Laplace (1985) and Souffrant et al. (1985). The relatively new Post-Valve T-Caecum (PVTC) cannulation technique has been reported as an alternative technique with the following advantages: an easy surgical procedure, less discomfort to the animal, a simple handling and minor negative affects on the physiological status of the animal (Van Leeuwen et al. (1988), Köhler et al. (1990,1991a,b). In order to evaluate the new PVTC cannula different comparisons with the re-entrant cannula, or the simple T-cannula, the ileo-rectal anastomosis and with results from literature have been carried out to investigate the comparability of the results measured with these different digesta collection techniques.

Materials & Methods

Experiment 1 The objective was to compare the ileal digestibilities of nutrients in four different diets (a control diet, a pectin-rich diet, a fibre-rich diet and a semi-synthetic diet) using pigs fitted with different types of cannulas according to different cannulation techniques (ileo-caecal re- entrant cannula, simple T-cannula and post-valve T-caecum (PVTC) cannula). Details of the experimental procedure are given elsewhere (Köhler et al. 1990).

Experiment 2 The ileal nutrient digestibilities of three different feedstuffs (maize, groundnut meal and sunflower meal) were measured using the PVTC technique. The results obtained were compared with corresponding results from the literature in which the re-entrant cannulation, the simple T-cannulation or the ileo-rectal anastomosis (IRA) technique were used. Complete details of the experiment are given by van Leeuwen et al. (1991).

Experiment 3 Digestibility measurements in PVTC cannulated pigs were compared with results obtained in pigs surgically modified according to the ileo-rectal anastomosis (IRA) technique. Additional details of this experiment have been given by Köhler et al.(1991a,b).

Diets The composition of the experimental diets of each experiment are presented in Table 1, 2 and 3.

Table 1. COMPOSITION OF EXPERIMENTAL DIETS (%) (EXPERIMENT 1)

	DIETS			
INGREDIENTS	CONTROL DIET	PECTIN -RICH DIET	FIBRE -RICH DIET	SEMI SYNTHETIC DIET
MAIZE	23.6	25.9	23.8	
BARLEY	19.0	8.0	12.3	
WHEAT	30.0	15.0		
SOYBEAN MEAL	18.4	18.1	18.1	
MOLASSES	5.0	5.0	5.0	
POTATO PULP		10.2		
BEET PULP		10.2		
APPLE PECTIN		4.0		
OATS			15.0	
OATHUSK MEAL			10.0	
ALFALFA			9.0	
WHEAT STRAW MEAL			3.3	
SOYA ISOLATE				22.3
WHEAT STARCH				28.2
CORN STARCH				28.2
ARBOCEL B 800[1]				6.0
SOYAOIL				4.0
ANIMAL FAT				4.0
L-LYSINE	0.13	0.19	0.06	
DL-METHIONINE		0.07	0.04	0.07
PREMIX[2]	1.37	1.37	1.37	1.37
$CaHPO_4*2H_2O$	1.50	1.44	1.30	
$Ca(H_2PO_4)*H_2O$				2.40
$CaCO_3$	0.97	0.50	0.79	1.00
$KHCO_3$				1.50
Cr_2O_3	0.05	0.05	0.05	0.50
TiO_2				0.50

[1] 100% CELLULOSE

[2] CONTRIBUTED THE FOLLOWING VITAMINS AND MINERALS PER KG OF DIET: RETINOL, 9000 IU; CHOLECALCIFEROL, 1800 IU; α-TOCOPHEROL, 40 mg; MENADIONE, 3 mg; RIBOFLAVIN, 5 mg; COBALAMINE, 40 µg; NICOTINIC ACID, 30 mg; D-PANTOTHENIC ACID, 12 mg; CHOLINE CHLORIDE, 150 mg; ASCORBINE ACID, 50 mg; KJ, 500 µg; $CoSO_4*7H_2O$, 2.5 mg AND Na_2SeO_3, 0.2 mg, NaCl, 3 g; $FeSO_4*7H_2O$, 0.40 g; $CuSO_4*5H_2O$, 0.1 g; MnO_2, 0.07 g; $ZnSO_4*H_2O$, 0.2 g. THIS MIXTURE ALSO SUPPLIED 20 ppm VIRGINIAMYCIN TO THE DIET.

Results and Discussion

Experiment 1 The apparent ileal digestibilities which have been obtained in the 1st experiment are presented in table 4. All ileal digestibility results were based on 100% Cr-recovery. Except for nitrogen and NDF in the fibre-rich diet and for nitrogen in the semisynthetic diet there were no differences between the treatments in ileal digestibilities of dry matter, nitrogen, crude fibre, acid detergent fibre (ADF) and neutral detergent fibre (NDF). In addition, the difference between the PVTC and re-entrant method obtained for nitrogen in the semisynthetic diet was statistically significant but of small magnitude. For the fibre-rich diet an influence of the collection technique on the digesta flow can be assumed. Although the re-entrant cannulation technique has been considered to be a reliable quantitative

TABLE 2. COMPOSITION OF EXPERIMENTAL DIETS (EXPERIMENT 2)

DIETS	1	2	3
MAIZE	95.3	70.8	71.0
GROUNTNUT MEAL EXPELLER		25.0	
SUNFLOWER MEAL SOLV. EXTR.			25.0
CR_2O_3	0.1	0.1	0.1
MINERAL MIXTURE[1]	3.6	3.1	2.9
VITAMIN AND TRACE ELEMENT MIXTURE[2]	1.0	1.0	1.0
ANALYSES:			
ORGANIC MATTER	82.7	83.2	82.5
CRUDE PROTEIN	8.3	19.1	13.6

[1] CONTRIBUTED THE FOLLOWING MINERAL SOURCES PER KG OF DIET: $Ca(H_2PO_4)_2*H_2O$, 12.5 g; NaCl, 5.0 g; $NaHCO_3$, 3.9 g; $KHCO_3$, 7.0 g; $FeSO_4*7H_2O$, 0.5 g; $CuSO_4*5H_2O$, 0.5 g; MnO_2, 0.05 g; $ZnSO_4*H_2O$, 0.2 g.THIS MIXTURE ALSO SUPPLIED 20 ppm VIRGINIAMYCIN TO THE DIET.
DIET 1 WAS ADDITIONAL SUPPLEMENTED WITH 2.5 g $Ca(H_2PO_4)_2*H_2O$ AND 5 g $KHCO_3$;
DIET 2 WAS ADDITIONAL SUPPLEMENTED WITH 2.5 g $Ca(H_2PO_4)_2*H_2O$ PER KG OF DIET.
[2] CONTRIBUTED THE FOLLOWING VITAMIN SOURCES AND TRACE ELEMENTS PER KG OF DIET:
VITAMIN A, 9000 IU; VITAMIN E, 40 mg; RIBOVLAVIN, 5 mg; NIACIN, 30 mg; D-PANTOTHENIC ACID, 12 mg; CHOLINE CHLORIDE, 150 mg; VITAMIN B12, 0.04 mg; VITAMIN D3, 1800 IU; VITAMIN K3, 3 mg; KJ, 0.5 mg; $CoSO_4*7H_2O$, 2.5 mg.

TABLE 3. COMPOSITION OF THE EXPERIMENTAL DIET (%) (EXPERIMENT 3)

INGREDIENTS	
MAIZE	3.95
BARLEY	6.00
WHEAT	10.50
SOYBEAN MEAL	22.50
MOLASSES	4.00
POTATO PULP	9.20
BEET PULP	10.00
ANIM. FAT	1.00
L-LYSINE HCL	0.18
METHIONINE DL	0.12
VITAMINS AND MINERALS[1]	1.00
$Ca(H_2Po_4)*H_2O$	1.50
$CaCo_3$	0.50
NaCl	0.30
Cr_2O_3	0.25

[1] CONTRIBUTED THE FOLLOWINF VITAMIN AND MINERAL SOURCES PER KG OF DIET: RETINOL, 9000 IU; CHOLECALCIFEROL, 1800 IU; α-TOCOPHEROL, 40 mg; MENADIONE, 3 mg; RIBOFLAVIN, 5 mg; COBALAMINE, 40 μg; NICOTINIC ACID, 30 mg; D-PANTOTHENIC ACID, 12 mg; CHOLINE CHLORIDE, 150 mg; ASCORBINE ACID, 50 mg; KJ, 500 μg; $CoSO_4*7H_2O$ 2.5 mg; Na_2SeO_3, 0.2 mg; $FeSO_4*7H_2O$, 0.40 g; $CuSO_4*5H_2O$, 0.1 g; MnO_2, 0.07 g; $ZnSO_4*H_2O$, 0.2 g. THIS MIXTURE ALSO SUPPLIED 20 ppm VIRGINIAMYCIN TO THE DIET.

Table 4. THE APPARENT ILEAL DIGESTIBILITIES OF DRY MATTER, NITROGEN, CRUDE FIBRE, ADF AND NDF FOR EACH DIET AND FOR EACH TYPE OF CANNULA (CALCULATED TO 100% CR RECOVERY)

Diet	Collection technique		
	PVTC cannula	T-cannula	Re-entrant
Control diet			
DM	73.4 ±0.32	73.0 ±0.54	73.3 ±0.57
Nitrogen	79.6 ±0.43	78.8 ±0.46	78.6 ±0.82
Crude fibre	8.4 ±2.34	10.1 ±1.97	10.6 ±1.96
ADF	18.2 ±1.74	19.5 ±1.29	19.1 ±1.09
NDF	30.5 ±1.37	31.8 ±1.20	31.1 ±1.34
Pectin-rich diet			
DM	58.0 ±0.55	56.6 ±1.99	57.5 ±1.72
Nitrogen	69.6 ±1.19	70.5 ±1.56	69.9 ±1.84
Crude fibre	- 1.0 ±2.00	-1.6 ±4.58	- .6 ±4.33
ADF	6.9 ±1.55	6.4 ±3.78	5.2 ±4.87
NDF	21.3 ±1.19	19.7 ±3.68	19.2 ±3.71
Crude fibre-rich diet			
DM	57.1 ±0.98	54.4 ±1.64	55.0 ±0.03
Nitrogen	71.6 ±1.07[a]	66.1 ±3.25	67.1 ±1.15[b]
Crude fibre	5.3 ±1.20	4.3 ±4.92	1.4 ±2.42
ADF	5.4 ±1.03	5.2 ±4.79	2.7 ±2.20
NDF	11.9 ±1.20[a]	11.2 ±4.43	7.7 ±1.17[b]
Semi synthetic diet			
DM	86.8 ±0.37		86.2 ±0.31
Nitrogen	92.4 ±0.30[a]		91.4 ±0.28[b]
Crude fibre	- 4.5 ±1.71		-5.2 ±1.76
ADF	2.0 ±2.01		0.6 ±2.04
NDF	1.3 ±1.34		0.0 ±1.38

ab MEANS (± SE) IN THE SAME ROW WITH DIFFERENT SUPERSCRIPTS DIFFER ($P < .05$)

collection method the recovery rate of Cr (added to the diet as Cr_2O_3 as a marker for the liquid phase) was considerable below 100% and also lower than the recovery rate of Co which was supplied to the diet as Co-EDTA. With the exception of the semisynthetic diet marker recoveries were also lower for the PVTC cannulated pigs. However, with the semisynthetic diet and the PVTC pigs the Cr- and Co-recoveries were higher than 100% and higher ($P< .05$) than in re-entrant cannulated pigs (Köhler et al. 1990). In re-entrant pigs the lowest Cr-recovery was found in the fibre-rich diet. In addition blockages in front of the ileal cannula and leakages around the cannula could be observed in these animals when the fibre-rich diet was fed. Both observations indicate a separation of the solid and the liquid phase and may explain the lower digestibilities calculated for the re-entrant cannulated animals.

Experiment 2 The apparent ileal digestibility of crude protein and lysine in three different feedstuffs measured according to the PVTC cannula were compared with corresponding results from the literature (Table 5). In general results measured in PVTC cannulated pigs were comparable with results reported in literature.

Table 5 THE APPARENT ILEAL DIGESTIBILITY (%) OF CRUDE PROTEIN AND LYSINE IN MAIZE, GROUNDNUT MEAL AND SUNFLOWER MEAL MEASURED IN PIGS FITTED DIFFERENT DIGESTA COLLECTION TECHNIQUES. MEANS ± STANDARD DEVIATION.[a]

	DIGESTA COLLECTION TECHNIQUE			
	PVTC CANNULA	RE-ENTRANT CANNULA	SIMPLE T-CANNULA	ILEO-RECTAL ANASTOMOSIS
MAIZE				
REFERENCES[b]	1	2		3
NUMBER OF BATCHES	1	6		3
CRUDE PROTEIN	65	67 ±4		73 ±7
LYSINE	51	54 ±8		58 ±7
GROUNDNUT MEAL EXPELLER				
REFERENCES	1		6	3
NUMBER OF BATCHES	1		3	1
CRUDE PROTEIN	80		73 ±3	85
LYSINE	76		66 ±3	82
SUNFLOWER MEAL SOLV. EXTR.				
REFERENCES	1	2	4, 5, 6	3
NUMBER OF BATCHES	1	1	4	4
CRUDE PROTEIN	75	74	73.1 73.0 74.0	75 ±3
LYSINE	74	73	78.9 78.0 73.0	75 ±3

[a] Van Leeuwen et al. 1991

[b] REFERENCES

1 = VAN LEEUWEN ET AL. 1991
2 = VAN LEEUWEN ET AL. 1989
3 = GREEN ET AL. 1987
4 = JØRGENSEN ET AL. 1984
5 = JUST ET AL. 1985
6 = KNABE ET AL. 1989

Experiment 3 The results of the 3rd experiment are summarized in Table 6. Except for Thr all amino acid digestibilities measured 3 weeks after surgery were lower ($P < .05$ - $P < .001$) in the IRA pigs as compared to the PVTC pigs. 9 weeks after surgery differences were found for DM, HIS, ILE, Met, Na and K. Results obtained in the last collection period show differences for DM, Ile, Met, Tre, Na and K. ($P < .05$ - $P < .001$). All results indicate a clear influence of the collection technique on digestibilities. In digesta of IRA pigs a considerable higher concentration of volatile fatty acids ($P < .001$) and of diaminopimelic acid (DAPA) ($P < .05$) was measured (Köhler et al. 1991a).This indicated an increased fermentation in digesta of IRA pigs and resulted in an increased dry matter digestibility ($P < .05$ - $P < .001$). Bacterial activity and the synthesis of bacterial protein may account for the differences in the ileal digestibility of amino acids. The missing hind gut digestion in IRA pigs had a clear influence on the digestibility of sodium and potassium. While in IRA pigs the sodium digestibility was about -10 until -20% it was about -650 until -700% in PVTC pigs which is in accordance with results reported in the literature (Drochner, 1984; den Hartog et al., 1988; Partridge, 1978; Partridge et al., 1986). The low sodium concentration in digesta of IRA pigs may be a result of a decreased sodium secretion into the intestinal lumen or an increased sodium reabsorption in the rectum. An increased sodium reabsorption and the minimized sodium excretion with the urine (Köhler et al. 1991b) indicate an increased aldosterone activity which would also explain the lower potassium digestibility which was found in IRA pigs.Potassium digestibility observed in PVTC pigs was similar with results found in literature.

Table 6 AVERAGE APPARENT DIGESTIBILITY OF DRY MATTER, NITROGEN, AMINO ACIDS, SODIUM AND POTASSIUM IN PIGS FITTED WITH ILEO-RECTAL ANASTOMOSIS (IRA) OR POST-VALVE T-CAECUM (PVTC) CANNULA 3, 9 AND 12 WEEKS POST-SURGERY (MEANS AND SE).

	3 WEEKS		9 WEEKS		12 WEEKS	
	IRA	PVTC	IRA	PVTC	IRA	PVTC
DM	64.3 ±1.1[a]	60.8 ± 0.6[b]	64.1 ±1.2[a]	59.7 ± 0.6[b]	69.9 ±0.7[e]	60.8 ± 0.4[f]
N	65.7 ±0.9[a]	69.0 ± 0.9[b]	66.4 ±1.0	69.3 ± 1.2	72.3 ±0.9	71.6 ± 0.6
ARG	82.2 ±0.8[e]	85.1 ± 0.6[f]	82.1 ±0.5	83.2 ± 1.2	84.3 ±0.3	84.6 ± 1.3
HIS	74.8 ±1.3[a]	79.0 ± 0.6[b]	77.2 ±0.7[c]	80.6 ± 0.6[d]	78.7 ±1.1	80.5 ± 0.6
ILE	75.1 ±0.6[e]	79.2 ± 0.7[f]	75.2 ±0.6[a]	78.1 ± 1.3[b]	77.2 ±0.6[a]	79.8 ± 0.6[b]
LEU	78.5 ±0.7[e]	81.6 ± 0.6[f]	79.1 ±0.7	81.2 ± 1.1	81.5 ±0.5	82.9 ± 0.5
LYS	70.0 ±1.1[a]	73.5 ± 0.9[b]	70.9 ±1.0	70.3 ± 1.2	66.9 ±0.9	68.2 ± 1.3
MET	83.5 ±0.7[c]	87.4 ± 0.7[d]	82.3 ±0.5[e]	88.1 ± 1.3[f]	83.8 ±0.6[e]	87.9 ± 0.7[f]
PHE	77.8 ±0.6[c]	80.9 ± 0.6[d]	78.1 ±0.7	80.5 ± 0.9	80.6 ±0.5	81.1 ± 0.6
THR	66.1 ±1.2	67.6 ± 0.9	67.9 ±1.2	66.9 ± 1.3	72.7 ±0.9[a]	70.0 ± 0.9[b]
VAL	71.6 ±0.7[a]	74.5 ± 0.8[b]	72.1 ±0.9	73.6 ± 1.2	75.7 ±0.7	75.7 ± 0.7
Σ AAS	75.5 ±0.8[a]	78.2 ± 0.6[b]	76.6 ±0.7	77.5 ± 0.9	79.0 ±0.7	78.6 ± 0.6
NA	-23.0 ±5.2[e]	-640.4 ±21.8[f]	-29.1 ±5.5[e]	-632.2 ±13.0[f]	-8.1 ±3.5[e]	-699.6 ±20.7[f]
K	25.7 ±2.8[e]	62.8 ± 1.1[f]	26.6 ±3.2[e]	59.8 ± 4.9[f]	36.9 ±2.6[e]	64.0 ± 2.2[f]

ab MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER P< .05
cd MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER P< .01
ef MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER P< .001

Conclusion Although there were some differences in general the ileal digestibility coefficients obtained with the PVTC method were comparable with those obtained with the re-entrant or simple T-cannulas. The results obtained with the PVTC and the IRA technique were different. There are clear indications that the IRA technique gives unrealistic results regarding the ileal digestibility of dry matter, nitrogen, amino acids and minerals.

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PRECAECAL NUTRIENT DIGESTIBILITY AND AMINO ACID ABSORPTION IN PIGS WITH ILEORECTAL ANASTOMOSES AND ILEOCAECAL RE-ENTRANT CANNULAE

U. Hennig, J. Wünsche, W.-B. Souffrant and F. Kreienbring

Research Centre of Animal Production, Institute of Animal Nutrition "Oskar Kellner", Rostock, Germany

Abstract

The apparent precaecal nutrient digestibility and amino acid (a.a.) absorption of 10 diets were estimated in pigs with end-to-side ileorectal anastomoses (IRA) and with ileocaecal bridge cannulae (ICB). The digestibility coefficients (d.c.) of organic matter, crude carbohydrates and nitrogen free extract were significantly higher in IRA pigs than in ICB pigs in two, three resp. four diets. There were no practically important differences for the d.c. of crude protein and crude fat in all diets. Of in the whole 180 a.a. absorption comparisons only 20 (11 %) showed differences larger than 5 %-units. The absorption rates of several a.a. were lower in IRA than in ICB pigs and of some other a.a. on the contrary. The methionine absorption of five legume as well as barley+lysine diets were up to 15 % resp. 4 %-units lower. This was due to the activity of microbes in the rectum. In order to minimize such a falsification it is proposed to make an end-to-end ileorectostomy. The deviations of other a.a. can partly be explained by differences in the a.a. pattern of endogenous protein, as it is shown in the case of feeding protein-free diets.

Keywords: digestibility, absorption, nutrients, amino acids, endogenous protein, ileum, methods, pig

Introduction

The evaluation of feed protein and the calculation of the a.a. contents in pig diets should be done on the base of precaecal a.a. absorption. Recently average values have been calculated and tabulated with mean d.c. values originating from various literature sources and described methods (Anonym, 1988; Anonym, 1989). The aim of this study was to compare the precaecal nutrient digestibility and a.a. absorption in pigs with IRA and ICB to supplement the investigations of Picard et al. (1984), Laplace et al. (1985) or Köhler et al. (1990).

Diets and animals

The composition of the experimental diets is shown in table 1. The diets 1 to 7 were calculated in order to meet the maintenance requirements of protein, a.a. and energy for pigs with 100 kg live weight (l.w.). In pigs weighing 50 to 60 kg the diets 8 and 9 were calculated for obtaining a moderate growth. DL-methionine was supplied in diets 3 to 6 to

prevent deficiency. This DL-methionine supplementation was not considered in the absoprtion calculations. In the same manner the L-lysine·HCl absorption in diet 10 was assumed to be 100 %.

Diets 1 to 7 were offered in each case to 5 ♀ IRA pigs weighing 107 to 126 kg and to 2 to 4 ♀ ICB pigs with 96 to 101 kg l.w.. The DM intakes amounted to 1600...1850 g/day and animal, that means 13...19 g DM/kg l.w., as it is proposed in the guide of Schiemann (1981). His feeding scale was also considered in diets 8, 9 and 10 with 6 ♀ growing IRA pigs 51 to 64 kg l.w.) or with 3 ♀ ICB pigs (101 kg l.w.). For the determination of the ileal endogenous protein 6 ♀ ileorectostomized pigs with 144 kg l.w. were given 2446 g DM of a protein-free mixture per day and one collection per animal for 4 days. The manner of digesta collection was quite different in IRA and ICB in diets 1 to 10: there was one collection per animal for 6 days or up to 4 collection periods for 12 hours around the clock with a one day break after the second period resp.

Table 1. Composition of experimental diets (g/kg DM).

Diet-No.	Ingredients
1	795 Barley 1 + 185 Yellow lupin 1 + 20 Mineral mixture*) (Min.m.)
2	720 Barley 1 + 260 Lupin 2 + 20 Min.m.*)
3	403 Lupin 1 + 571.5 protein-free mixture (p.f.m.) + 25 Min.m.*) + 0.5 DL-Methionine(Met)
4	594 Lupin 2 + 377.6 p.f.m. + 25 Min.m.*) + 3.4 Met
5	603 Field bean 1 + 368 p.f.m. + 25.6. Min.m.*) + 3.4 Met
6	608 Field bean 2 + 364 p.f.m. + 25 Min.m.*) + 3 Met
7	489 Rapeseed meal + 486 p.f.m. + 25 Min.m.*)
8	327 Barley 1 + 581 Wheat + 77 Fish meal 1 + 10 Min.m. + 5 Vitamine mixture for piglets (Vit.m.)
9	329 Barley 2 + 581 Wheat + 75 Fishmeal 2 + 10 Min.m. + 5 Vit.m.
10	980.8 Barley 2 + 10 Min.m. + 4.2 L-Lysine·HCl + 5 Vit.m.
11	787 boiled potato starch + 98.4 Sucrose + 49.2 Cellulose powder + 49.2 Rapeseed oil + 16.2 Min.m.

*)Vit.m. "Summavit ® forte": 1 dragee per day

Results and Discussion

In some diets the d.c. (%) of nutrients was significantly higher in IRA than in ICB: for organic matter in diet 4 70/64 (IRA/ICB) and 5 83/75, crude carbohydrates in diets 1 76/69, 4 66/59 and 5 84/75, nitrogen-free extract in diets 1 83/77, 2 81/74, 4 76/67 and 5 90/82. These differences can be caused by the absence of the ileocaecal-valve (no temporal stowage of chyme in the ileum) in ICB and/or by the additional stowage of chyme in the rectum of IRA pigs. In spite of that, there were no practical important differences in d.c. of crude protein in all diets: 1 77/75, 2 75/77, 3 84/86,

4 82/80*), 5 78/73, 6 80/76, 7 73/68, 8 81/81, 9 80/78 and 10 74/70 and the same for the d.c. of crude fat: 1 57/63, 2 59/65, 3 83/88, 4 74/80*), 5 88/84, 6 88/85, 7 82/83, 8 74/73, 9 70/70 and 10 62/62 (* = significance α = 0.05).

For the a.a. absorption some remarkable differences exist between the two methods as it is shown in table 2.

Table 2. Precaecal absorption rates (%) of amino acids.

Diet-No.	Ile IRA/ICB	Lys IRA/ICB	Met IRA/ICB	Cys IRA/ICB	Thr IRA/ICB	Trp IRA/ICB
1.	75/77	71/76*	72/81*	85/83	72/69	76/69
2.	74/80	72/81*	74/81*	84/80	70/74	74/71
3.	82/86	82/86	75/85*	88/80	82/84	78/78
4.	83/83	85/86	75/77	87/82*	81/81	75/66*
5.	75/73	77/78	64/74*	75/77	72/65	65/62
6.	76/75	80/81	60/75*	76/74	74/69	71/63
7.	73/71	74/75	76/80	76/75	72/69	66/65
8.	80/85	81/82	87/88	83/78*	77/76	85/82*
9.	82/83	78/78	87/85	82/81	74/73	78/74
10.	79/74	82/79	78/82*	78/77	68/61*	62/50

*Significance α = 0.05

In five legume diets and in the barley+lysine diet the methionine absorption was significantly higher in ICB than in IRA pigs: the differences are 9, 7, 10, 15 and 4 %-units in the diets 1, 2, 3, 5 and 10 resp. Absorption differences for lysine were found in diet 1 and 2 (5 and 9 %-units resp.). The intake levels particularly of methionine in a protein-bound form in the legume diets were very small and therefore already slight bacterial activities in the digesta are able to reduce the absorption rate and to enhance the excretion rate to a high degree. In connection with that the carbohydrate digestion in the small intestine was not so high in these diets than in other diets and more fermentable substances are available in the rectal stowage room. It can be concluded that it is possible to minimize this falsification by means of the end-to-end ileorectostomy as it was done by Green et al. (1987). On the other hand, the absorption rates of cystine, threonine and tryptophan were higher in IRA than in ICB pigs.
When all a.a. with significant absorption differences will be classified in IRA lower (I) and IRA higher (II) than ICB, as it is done in table 3, then it is a remarkable picture. Group I encloses methionine, lysine, isoleucine, valine and alanine, group II includes phenylalanine, cystine, tryptophan, threonine, arginine and histidine but also glutamic acid and glycine. Aspartic acid and proline appear in both groups. For several a.a. it is assumed that protein and a.a. of endogenous origin seem to be responsible for this picture. Therefore the average endogenous a.a. and CP excretion in the digesta of IRA pigs should be compared with mean values from

cannulated pigs (in mg per 100 g DM intake). This comparison is given in table 4 in columns 2 and 3.

Table 3. Amino acids with significant absorption differences.

I. IRA lower than ICB	Diet-No.	II. IRA higher than ICB	Diet-No.
Methionine	1, 2, 3, 5, 6, 10	Phenylalanine	1, 4, 8, 10
		Cystine	4, 8
Lysine	1, 2	Tryptophan	4, 8, 10
Isoleucine	2, 8	Threonine	10
Leucine	2	Arginine	10
Valine	2	Histidine	10
Alanine	2		
		Glutamic acid	4
		Glycine	4, 9
Aspartic acid	2	Aspartic acid	10
Proline	8	Proline	5

Table 4. Average amounts of endogenous amino acids and crude protein in the digesta of IRA and cannulated pigs (mg/100 g DM intake of protein-free diets).

	1. IRA (n = 6)	2. IRA	n	3. Cannulae	2. in% of 3.
Arg	29.5 ± 2.8	28.7 ± 5.6	8	44.1	65
His	12.5 ± 2.4	14.5 ± 2.1	8	17.9	81
Ile	33.2 ± 5.4	26.8 ± 4.6	8	24.3	110
Leu	48.6 ± 6.6	45.1 ± 9.1	8	49.6	91
Lys	46.1 ± 9.3	39.0 ± 10.4	8	37.8	103
Met	11.5 ± 2.6	13.4* ± 2.2	3	10.4	129*;56
Cys	14.2 ± 1.3	12.9 ± 2.2	8	20.5	63
Phe	30.5 ± 7.5	26.8 ± 5.1	8	32.1	83
Tyr	19.4 ± 3.8	21.0 ± 2.4	7	25.3	83
Thr	33.9 ± 5.4	39.6 ± 8.8	8	50.8	78
Trp	10.9 ± 1.8	10.9 ± 1.8	1	18.3	60
Val	37.2 ± 5.8	35.9 ± 4.2	8	40.0	90
Ala	49.9 ± 10.0	42.2 ± 11.0	8	51.0	83
Asp	62.5 ± 9.1	58.0 ± 9.7	8	78.0	74
Glu	82.0 ± 12.2	73.3 ± 10.4	8	82.9	88
Gly	45.0 ± 9.8	55.2 ± 13.4	8	118.3	47
Pro	79.1 ± 66.1	77.2 ± 12.6	4	251.0	31
Ser	29.9 ± 4.8	35.5 ± 7.8	8	46.8	76
CP (N×6.25)	841 ±159	872 ±137	8	1387	63

1. Own results*, 2. Averages calculated from column 1. plus beneath cited literature: Souffrant et al. (1985)*; Pietruschka (1987)*; Green et al. (1987); Green and Kiener (1989); Mariscal-Landin et al. (1990);*End-to-side anastomoses; Met 5,8 ± 0,8 (n = 5) ≙ 56 % in End-to-end anastomoses; 3. Wünsche et al. (1987)

Of the essential a.a. arginine, histidine, cystine, threonine and tryptophan the excretion levels in IRA are smaller than in ICB pigs. The apparent absorption rates of these a.a. are higher in IRA than in ICB pigs in one or another diet (see table 3). Similar reactions were observed in aspartic acid and glycine. On contrary the excretion levels of endogenous isoleucine and methionine are increased in IRA with the result that the apparent absorption rates are decreased. Under the conditions of protein intake the a.a. of endogenous origin probably interact the absorption rates of several native a.a.. But in general these influences should not be overevaluated. From all significant absorption differences of each group, presented in table 3 the differences were only in 20 cases equal or larger than 5 %-units. This is 11 % of the conducted 180 comparisons. The number of observed deviations is relatively small. Therefore the a.a. absorption rates available from the literature and estimated by both methods should be tabulated. But the methionine absorption rate which were estimated with end-to-side IRA pigs in diets with a low content of protein-bound methionine must be excluded. Two additional facts from the present study are interesting to know. Firstly, the methionine excretion levels in protein-free diets using end-to-side IRA pigs (Souffrant et al., 1985; Pietruschka, 1987; our own findings) were double as high as in end-to-end IRA pigs (Green et al., 1987; Green and Kiener, 1989; Mariscal-Landin et al., 1990). Secondly, the excretion level of endogenous a.a. and CP in our own experiment with 144 kg l.w. pigs was nearly equal to those in the trials of the other research workers, who partly used little pigs (20 to 40 kg l.w.). It can be concluded that the a.a. pattern of ileal endogenous protein and also the excretion amounts per 100 g DM intake during protein free nutrition are more or less independent of the ontogenesis in pigs.

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ASSESSING THE PROPORTION OF BACTERIA NITROGEN IN FAECES AND DIGESTA OF PIGS USING DAP ESTIMATION AND BACTERIA FRACTIONATION

J. Wünsche, T. Völker, E. Borgmann, W.-B. Souffrant

Research Centre of Animal Production Dummerstorf-Rostock
Division of Animal Nutrition 'Oskar Kellner', Rostock, Germany

Abstract

In faeces samples of intact (INT) pigs as well as in ileal digesta samples of surgically differently prepared pigs bacterial fractionations and 2.6-diaminopimelic acid (DAP) estimations were conducted in order to calculate the bacterial nitrogen proportions in faeces N and digesta N. These calculations were done in two different ways, 1. using the premise that all the nitrogen in the 'bacterial fraction C' is only of bacterial origin or 2. putting up the premise that DAP found in fraction A originates from intestinal bacteria adhering to feed particles. The following proportions of bacteria N in per cent of total N were ascertained by the two calculation procedures after feeding various diets: faeces of INT pigs: 43.0...68.2 or 69.6...89.0; digesta of operated pigs - ileorectostomized, colon descendens open (IRAo) 22.3...57.0 or 46.2...73.8; re-entrant cannulated (REC) 17.0...35.7 or 25.2...53.6; ileorectostomized, colon descendens closed (IRAg) only 3 pigs on one diet 23.6 or 24.2. In the digesta N of protein free fed IRAo pigs only 22.0 or 22.6 % was bacteria N. Though there was a high individual variability and a large analytical variation width too, the results got with REC and IRAg pigs suggest a bacteria N proportion of approximately 25 per cent of the total N in ileal digesta.
Keywords: pigs, ileal digesta, faeces, bacterial fractionation, DAP, bacteria nitrogen

Introduction

Recent investigations on protein digestibility and amino acid absorption in various parts of the pigs' digestive tract led to the knowledge that amino acid absorption has finished at the terminal ileum. Most of the nitrogenous compounds getting into the large intestine are microbiologically fermented and no longer usable to the animal for protein synthesis. About 60 to 90 per cent of the excreted faecal N of pigs is bacteria N (Laplace et al., 1985; Mauritz-Boeck et al., 1986; Wünsche et al., 1987). Though the pig-own digestive enzymes are responsible to about 90 per cent for total digestion (Bölduan & Jung, 1982), the intestinal bacteria also have a great influence on nutrient digestibility and nitrogen and energy metabolism (Meinl, 1978; Rerát, 1981). The fact of a high digestibility of ß-glucans in the pig small intestine

(Fadel et al., 1989) indicates a substantial bacterial activity in this part of the digestive tract. According to Graham et al. (1986) more than 10^7 Lactobacilli could be found per g fresh duodenal and ileal digesta. Hence microbial enzymes also play an essential role in precaecal nutrient digestion.
In order to enlarge the konwledge about the extent of bacterial protein synthesis in different parts of the digestive tract DAP-estimations and bacterial fractionations were done in samples of faeces and ileal digesta of intact (INT) and surgically differently prepared pigs, and bacteria N proportions in the excreta were calculated.

Experimental

Analytical methods

Samples of faeces or ileal digesta - taken immediately after excretion - were homogenized and partly preserved in 0.7 % formaldehyde, partly freeze dried. The preserved samples were fractionated according to the procedure of Stephen & Cummings (1980) in the modification given by Meinl & Kreienbring (1985). So an isolated, relatively clean bacteria fraction (fraction C) was got besides fraction A which essentially consisted of particles originating from the diets, and a small negligible fraction B containing very fine diet particles. As a bacteria marker 2.6-diaminopimelic acid (DAP) was analyzed in fractions C and A as well in the whole freeze dried samples of faeces and digesta. The analytical method of the DAP-estimation was carried out by column chromatography and is described in its methodical details by Völker et al. (1991).

Experimental animals and diets

A general view on the experimental pigs as well as the various diets, which had been used primarily for investigations of protein digestibility and amino acid absorption, is presented in tables 1 and 2.

Table 1. Experimental pigs, operation techniques and live weights

Pigs	Operation technique	Abbrev.	Live weights (kg)
Intact	-	INT	56...154
Cannulated	Ileo-caecal re-entrant cannulae	REC	59...103
Ileorecto-stomized	Ileo-rectal anastomosis with open colon descendens	IRAo	36...144
"	Ileo-rectal anastomosis with closed colon descendens	IRAg	53

Besides the normal INT pigs different operation techniques had been used for receiving ileal digesta. All techniques as there are re-entrant cannulation (REC) and fitting ileo-rectal anastomoses (IRA) with open colon descendens (IRAo), i.e. end-to-side anastomosis or closed colon descendens (IRAg), i. e. end-to-end anastomosis are comprehensively described by Hennig et al. (1990).
As can be seen from table 2, a proteinfree diet and some semi-synthetic, cereal and mixed-feed diets were examined by one, two or three of the four pig categories.

Table 2. Diets fed to experimental pigs.

Diets (Type)	number (n)	Pigs, number (n) INT	REC	IRAo	IRAg
1. Proteinfree mixture[1)]	1	-	-	3	-
2. Protein concentrate[2)]+ proteinfree mixture	4	-	7	19	-
3. Barley+lysine	3	11	-	11	-
4. Rye+casein	1	7	-	4	-
5. Barley+canola	1	3	-	-	-
6. Wheat+barley+fishm.	2	6	5	12	-
7. Commercial mixed feed	1	4	-	4	3

1) g/kg DM: 800 steamed potato starch+100 sucrose+50 cellulose+50 oil

2) Field bean; lupin; canola; fishmeal

Results and Discussion

In table 3 the DAP values in bacteria fraction C (mg/g N) of faeces and ileal digesta are given.
Comparison with data from literature were only found out for pig faeces as well as rumen and duodenal content of cattle. The mean DAP content of 26.6 mg per g digesta bacteria N of IRAo pigs is within the variation width of faeces or rumen bacteria N. Although the variation width between the single animals is rather wide, differences between the 3 surgical methods are evident. The high DAP values in the digesta of IRAo pigs suggest that there are some possibilities of bacterial development and fermentation because of a possible reverse stream into the colon descendens. Reasons for individual differences may be a more or less dense bacteria population in the small intestine and occurence of bacteria species with variable DAP contents.

Calculations of the bacteria nitrogen proportion in per cent of excrement total N was done in two different ways. For both variants some premises had to be appointed:
1. As microscopic checkings of the bacteria fraction C showed it contains besides a high proportion of bacteria fine fibre particles, residuals of vegetable cell walls - consisting of structured carbohydrates and lignin -, which are

Table 3. DAP levels in isolated bacteria fractions of animal gut contents and faeces (own results in comparison with values from literature).

Animal species	Bacterial fraction, isolated from ...	mg DAP per g Bact.-N	Authors
Pig (INT)	Faeces	14.2...29.0	this paper
" (REC)	Ileal digesta	7.5...18.8	" "
" (IRAo)	" "	10.0...39.6	" "
" (IRAg)	" "	5.2... 7.3	" "
Pig	Faeces	16 ...24*)	Wünsche, Meinl et al. (1987)
"	"	37.5...39.8	Poppe & Meier (1983)
"	"	43 ...88	Meinl & Kreienbring (1985)
"	"	19.4/29.9	Laplace et al. (1985)
"	"	28/30*)	Mason et al. (1982)
Cattle	Rumen content	20 ...40	Whitelaw et al. (1984)
Cow	" "	34*)	Krawielitzki & Voigt (1988)
Young cattle	Duodenal digesta	46*)	Gabel & Poppe (1985)

*) Converted values

practical nitrogen free. Hence it follows that the whole N amount in fraction C is pure bacterial N. On this base calculated percentages of bacterial N in total N of pigs'faeces and digesta are given in table 4.

Table 4. Bacterial N in % of total N in faeces and digesta of pigs. Calculation base: N amount in bacterial fraction C related to the respective total N amount.

Diet type No.	Faeces INT pigs $\bar{x}$	Ileal digesta, pigs with ... REC $\bar{x}$	IRAo $\bar{x}$	IRAg $\bar{x}$
1	-	-	22.0	-
2	-	17.0...22.5	22.3...43.1	-
3	55.6...68.0	-	32.3...57.0	-
4	58.0	-	35.4	-
5	51.9	-	-	-
6	43.0	35.7	36.1...36.6	-
7	68.2	-	35.6	23.6
C.V.	2.2...12.0	14.4...46.4	9.3...42.9	21.4

This calculation manner provides bacterial N proportions in faeces of 43...68 %, which range - compared to values in literature - at the lower bound. They are, however distinctly higher than in digesta (17...57 %). The very low bacteria N share in the digesta N of the IRAg pigs suggests that this

surgical technique is suitable for getting unadulterated digesta, which corresponds largely to the not accessible digesta of intact pigs.

2. For another calculation procedure DAP is used as a bacterial marker. In this case the DAP content of fraction A must be taken into account, because it is supposed that this DAP originates from gut bacteria, which had not been removed from the coarse feed particles and therefore are adhering to them. This DAP content from fraction A - related to the same bacteria N proportion as in fraction C - and summed led to bacteria N proportions in the total N of faeces or digesta as presented in table 5.

Table 5. Bacterial N in % of total N in faeces and digesta of pigs. Calculation base : DAP amounts in the fractions C+A.

Diet type No.	Faeces INT pigs $\bar{x}$	Ileal digesta, pigs with ... REC $\bar{x}$	IRAo $\bar{x}$	IRAg $\bar{x}$
1	-	-	22.6	-
2	-	25.2...53.6	50.1...59.4	-
3	79.2...83.2	-	62.8...73.8	-
4	76.7...81.0	-	49.6	-
5	89.0	-	-	-
6	69.6	49.9	46.2...52.4	-
7	77.8	-	48.3	24.2
C.V.	3.2...12.0	12.4...30.9	8.0...42.1	21.2

These values are distinctly higher than those computed only from the N content of bacteria fraction C (cf. table 4). In faeces they range from 70 to 90 %, in ileal digesta from 46 to 74 %. In digesta of IRAg pigs (up to now 3 animals only) this percentage is again very low (24.2 %).
A better agreement between the two calculation procedures for the bacteria N level in pig excrements will be obtained by an improvement of the fractionation method, especially in using a more effective detergent solution for detaching bacteria from feed particles.

From all results it can be concluded that the variability of bacteria percentages in pig excrements is very high. Individual differences are at the terminal ileum higher than in faeces as could be seen from the coefficients of variation. Influences of feed ingredients could only be observed in tendency, e.g. higher DAP and bacterial N values in diets containing barley.
In order to avoid falsifications of bacteria content in digesta, a subsequent propagation of bacteria has to be obviated. Best surgical techniques for getting unchanged ileal digesta are the REC and the IRAg operation method. Results with so prepared pigs show bacteria N proportions in ileal digesta N not higher than 25 %.

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COMPARATIVE STUDIES ON DUODENAL, ILEAL AND OVERALL DIGESTIBILITY OF DRY MATTER, TOTAL PHOSPHORUS AND PHYTIC ACID IN PIGS USING DUAL-PHASE MARKERS

A.W.Jongbloed, P.A. Kemme and Z.Mroz

Research Institute for Livestock Feeding and Nutrition (I.V.V.O.), Lelystad

Abstract

Cr-NDR (4 $g \cdot kg^{-1}$) and Co-EDTA (5 $g \cdot kg^{-1}$) were used as dual phase markers in two diets based either on maize and soybean meal (MS) or on tapioca and maize gluten feed (TMG) for 6 barrows of about 37 kg live weight, fitted with two simple T-cannulae in the duodenum and terminal ileum to compare duodenal, ileal and overall digestibility of dry matter, total P and phytic acid.

Duodenal digestibility coefficients of these nutrients were always lower when calculated from the concentrations of Co, irrespective of the diet.

Ileal digestibility coefficients of dry matter, total P and phytic acid obtained for the MS diet were also markedly lower ($p<0.05$) with Co as a marker, whereas for the TMG diet the compared values were very similar for both markers.

Overall digestibility of dry matter from both diets was from 2.2 to 4.5 percentage units lower when using Co as a marker. Also, the relative values for total P were lower from 10.5 to 12.9 percentage units.

It seems, that by using Cr-NDR the apparent digestibilities of dry matter, total P and phytic acid measured in the duodenum were somewhat overestimated whereas by using Co-EDTA the relative measurements were underestimated.

Values of the duodenal, ileal and overall digestibility of the nutrients from both diets obtained from the quantitative collection of digesta and faeces were not known, and therefore the averages calculated on the base of both markers may be adopted as more representative.

Keywords: cannulated pigs, markers, apparent digestibility.

Introduction

Measurements of many digestion processes using re-entrant cannulated or ileo-rectal anastomized pigs are always affected by some disturbances of the peristaltic movement or abnormal functioning of the intestine (Laplace and Darcy, 1980; Hennig et al., 1986; Köhler et al.,1990).

On the other hand, it is recognized that by using pigs fitted with simple T-cannulae in the duodenum or terminal ileum, the measurements may not be truly representative for the digesta passing these points, because of a differential sampling of either the liquid or solid phase. Graham and Aman (1986) reported that the composition of both duodenal and ileal digesta varied considerably during the sampling period and was particularly influenced by feeding time.

To correct for the possibility of non-representative sampling of digesta from sheep with T-cannulae Faichney (1980) introduced the double-marker method.

However, in studies on pigs still mostly chromium (as Cr_2O_3 or Cr-NDR) is used as a single solid-phase marker, although contradictory results concerning its accuracy are available in the literature (Livingstone et al.,1980; Partridge et al.,1985; Graham et al.,1985).

Therefore, the objective of the present study was to compare the apparent duodenal,ileal and overall digestibilities of some nutrients (dry matter,

total phosphorus and phytic acid) in pigs fitted with simple T-cannulas, using dual-phase markers (Cr-NDR as a solid-phase marker and Co-EDTA as a liquid-phase marker).

Materials and Methods

Six Yorkshire x (Finnish Landrace x Dutch Landrace) barrows of about 37 kg live weight, fitted with two simple T-cannulae in the duodenum and terminal ileum, were fed two diets, i.e. based on maize and soybean meal (MS) or on tapioca and maize gluten feed (TMG) in two test periods, lasting for 28 days, according to a simple classification. Two daily rations were supplemented both with Cr-NDR (4 $g_i kg^{-1}$) as an indigestible marker for the solid phase and with Co-EDTA (5 $g \cdot kg^{-1}$) for the liquid phase and mixed with water at a ratio of 1:2.5 (w/v) just before feeding at 7 a.m. and 4 p.m. (Table 1).

Table 1. Ingredients of experimental diets and analysed dietary nutrients ($g \cdot kg^{-1}$)

Diet	MS	TMG
Ingredients		
Maize	859.50	-
Soybean meal	124.55	124.55
Tapioca	-	421.00
Maize gluten feed	-	336.00
Sunflower meal	-	80.00
Soya oil	-	26.00
Limestone	11.80	8.30
NaCl	2.50	2.50
Choline chloride	0.25	0.25
Trace min.-vit. premix*	1.40	1.40
Dietary nutrients		
Dry matter	863	882
Crude protein	136	157
Ca	5.1	5.5
Total P	3.3	4.1
Phytic acid	7.4	7.5

* Supplied ($mg \cdot kg^{-1}$ diet): 2.4 vit A, 0.04 vit. D_3, 8.0 vit. E, 4.0 riboflavin, 20.0 nicotinic acid, 8.0 panthotenic acid, 0.02 vit. B_{12}, 125.0 antioxidant, 430.0 $FeSO_4$, 50.0 MnO, 155.0 $ZnSO_4$, 40.0 $CuSO_4$, 2.0 KJ, 0.03 Se and 555.51 carrier.

The pigs were housed individually in pens of 2.00 x 1.45 m throughout the whole experiment.

During the test periods the feeding level was equivalent to 2.3 times maintenance requirement, i.e. 418 kJ ME $W^{0.75}$.

After 3 days of feeding the diets, samples of duodenal digesta were collected 5 times in 1 or 1.5 hour intervals (assuming maximally ca. 300 g of fresh material during the first two collections, 450 g during the third collection and quantitatively during the last two collections), beginning at 7 a.m. on the 4-th and the 6-th day of the test period.

Faeces were collected at random on the 9-th and 10-th day, whereas samples of ileal digesta were collected quantitatively on the 10-th, 12-th and 14-th day (7 times in 1-2 hour intervals, beginning at 7 a.m.).

The samples of digesta were collected into sterilized polyethylene bags attached to the cannula barrel, freeze dried and ground to pass a 1 mm sieve prior to analysis.

Analytical and statistical procedures of this experiment were described elsewhere (Simons et al.,1990).

Results and Discussion

Comparisons of the apparent duodenal digestibility coefficients of dry matter, total P and phytic acid calculated from the concentrations of Cr and Co are presented in Table 2.

Table 2. The apparent duodenal digestibility coefficients of dry matter, total P and phytic acid in pigs calculated from the concentrations of Cr and Co (in %).

Diet		MS		TMG	
		Cr-NDR	Co-EDTA	Cr-NDR	Co-EDTA
Dry matter	mean	5.9	-4.3	5.1	-16.3**
	SD	15.6	8.9	6.3	6.3
Total P	mean	7.8	-4.3	9.6	-10.0**
	SD	14.2	10.4	9.5	2.9
Phytic acid	mean	49.5	43.2	54.8	47.0
	SD	9.9	12.4	5.7	6.1

SD- standard deviation
** statistically significant at P<0.01

Irrespective of the treatment, all the values obtained with Co as a liquid phase marker were lower. Standard deviations were relatively high for both markers.

In studies of Low et al. (1978) mean dry matter digestibility in the duodenum of pigs with intestinal re-entrant cannulas varied from 4 to 6 percentage units. On the other hand, Żebrowska et al.(1983) reported that the mean duodenal output:dietary intake ratio of dry matter ranged from 1.03 to 1.05. The latter data seem to be more convincing, because the higher output than intake of dry matter is due to salivary and gastric secretions (4-8 kg/ 24 h), thus providing endogenous N and ash into the duodenum. Therefore, our results based on Cr supposingly overestimate the degree of dry matter digestibility in the duodenum, whereas the values obtained by using Co seem to be too low.

Duodenal digestibility of P was also lower when calculated from the concentration of Co, whereas the values of phytic acid degradation anterior

to the duodenal cannula were higher. Information on mineral digestion in different sections of the gastro-intestinal tract is still scarce. Partridge (1978) reported a slight net absorption of P anterior to the duodenal cannulae with a diet containing maize starch and sucrose, whereas this tendency was not observed with a diet composed of barley and fine wheat offal. It seems therefore, that there is an impact of different dietary factors.

The apparent ileal digestibility of dry matter, total P and phytic acid in pigs, calculated from the concentrations of Cr and Co is given in Table 3.

Table 3. The apparent ileal digestibility of dry matter, total P and phytic acid in pigs, calculated from the concentrations of Cr and Co (in %).

Diet		MS		TMG	
		Cr-NDR	Co-EDTA	Cr-NDR	Co-EDTA
Dry matter	mean	71.3	66.5*	51.3	51.7
	SD	3.0	5.2	9.6	7.3
Total P	mean	40.0	31.3*	30.5	31.4
	SD	7.7	6.9	15.2	10.1
Phytic acid	mean	38.6	30.7*	36.0	36.6
	SD	11.0	8.5	15.7	10.9

* statistically significant at $P < 0.05$

For the MS diet, apparent ileal digestibility coefficients of all the nutrients calculated on the base of Co concentration were markedly lower ($p<0.05$), whereas for the TMG diet the compared values were very similar for both markers. No clear explanation of this fact was found.

Overall apparent digestibility of dry matter and total P calculated with Co was lower, independently of the diet (Table 4).

Table 4. The apparent overall digestibility of dry matter and total P in pigs, calculated from the concentrations of Cr and Co (in %).

Diet		MS		TMG	
		Cr-NDR	Co-EDTA	Cr-NDR	Co-EDTA
Dry matter	mean	85.1	82.9*	81.4	76.9***
	SD	2.2	1.6	1.2	1.6
Total P	mean	33.0	22.5**	47.9	35.0***
	SD	4.1	4.9	4.7	7.2

However, evaluation which values are more accurate is difficult, and therefore it seems reasonable to suggest that an average of both markers ought to be adopted.

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ESTIMATION OF UNDIGESTED DIETARY PROTEIN BY THE USE OF ^{125}I-LABELLED PROTEIN

M. F. Fuller & A. Cadenhead
Rowett Research Institute
Aberdeen, AB2 9SB, U.K.

Abstract

To distinguish the endogenous amino acid losses in ileal digesta from undigested food protein the use of ^{125}I-labelled casein as a model food protein was evaluated. A small amount (2-3$_{\mu}$Ci) of labelled casein was added to a synthetic diet with 10% casein and given to pigs with cannulas in the terminal ileum. The flow rates of radioactivity and of nitrogen at the terminal ileum were measured. Whereas it was expected that the ratio of nitrogen to radioactivity in the ileal digesta would be higher than in the food due to the addition of endogenous nitrogen, the reverse was found, perhaps suggesting that iodination may have altered the inherent digestibility of the casein.

Introduction

In estimating the true pre-caecal digestibility of food proteins the nitrogen passing the terminal ileum may be considered to consist of two fractions, a component of undigested dietary protein and an endogenous component, which is conventionally assumed not to vary with the amount or composition of the diet fed. This endogenous component represents not the total endogenous secretion into the intestine but that fraction which is not reabsorbed. It would be of considerable value in the estimation of the real digestibility of food constituents to be able to distinguish these two components, but there are no direct means of doing so. Approaches based on the use of intrinsically labelled proteins in the diet (e.g. Partridge *et al.*, 1985) or on labelling the animal's secretions (e.g. de Lange *et al.*, 1990) may be criticised on the grounds that labelled amino acids absorbed by the enterocytes may be rapidly incorporated into secreted proteins (Alpers, 1983). To avoid this objection non-recyclable labels are needed to label dietary protein. Labelling with ^{125}I has been used to estimate the resistance to proteolysis of specific proteins. This approach was based on the premise that tyrosine labelled with ^{125}I could not be incorporated into endogenous proteins so that any peptide-bound labelled tyrosine reaching the terminal ileum must represent undigested dietary protein. It was therefore expected that the ratio of radioactivity to nitrogen would decrease during gut passage as endogenous proteins were added to the digesta and this 'dilution' could be used to estimate the amount of endogenous protein added.

Materials and Methods

A diet with casein (200 g/kg) and with chromic oxide (5 g/kg) as a marker was given twice daily to 3 pigs of approximately 54kg which had T-cannulas at the end of the ileum. Iodinated casein was prepared using Na^{125}I (Amersham International) and Iodo-beads (Pierce Chemical Co.). The iodinated casein (1 mole ^{125}I/250 moles tyrosine) was separated from free iodide by gel filtration using phosphate buffer containing KI. A small quantity (2-3 μCi) of freshly iodinated casein was added to three successive meals. All digesta were

collected for 8h after the last of these meals and homogenised. Nitrogen and chromic oxide were determined by standard methods. A sample of ca 3g of the homogenised digesta was taken for counting. Further 3g samples were homogenised with 3ml TCA and centrifuged. The precipitates were washed repeatedly with distilled water until no further counts were extracted. Up to six washings were generally needed. When $Na^{125}I$ was added to the digesta of a pig which had not been given labelled protein again some six washings were needed to reduce the counts in a TCA precipitate to background levels. Extraction was not increased by the addition of unlabelled KI. The radioactivity in the washed precipitates was counted.

Results

The proportion of the nitrogen intake which disappeared before the terminal ileum was 0.86 (SE O.023), whereas only 0.72 (SE 0.029) of the ^{125}I disappeared. Thus the expected dilution of the radioactivity by added endogenous nitrogen did not occur. The ratio of N: radioactivity (μgN/cpm) in the food was 5.41. Some of the radioactivity in the digesta was in the TCA-soluble fraction but even after repeated TCA extraction and washing the ratio of N: radioactivity in the TCA-precipitate remained lower than in the food (3.7; SED 0.814).

Discussion

Although iodinated proteins have been used to estimate the extent of degradation of specific proteins (e.g. Kilpatrick *et al.*, 1985) this approach does not seem to have been applied previously to the estimation of the endogenous contribution to digesta nitrogen. One explanation for our results is that the labelled casein was digested to a lesser extent than the unlabelled casein which formed the bulk of the dietary protein. The flow of nitrogen from the ileum of these pigs given casein as the sole dietary protein was 5.0g/d, similar to that determined on other occasions with the same diet (Wang & Fuller, 1989; Fuller & Cadenhead, 1991). Such values, taken in conjunction with estimates of nitrogen flow with protein-free diets, suggest that the true digestibility of the casein is much higher, and is probably digested almost completely. This highlights the extent to which the iodinated casein survived. The results suggest that, despite the very low level of labelling (1 mole ^{125}I/250 moles tyrosine) iodinated proteins may be distinguished in digestion amd may therefore not be suitable for such studies.

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EVALUATION OF CHROMIC OXIDE WITH LOWER CONCENTRATION AND OF HCL-INSOLUBLE ASH AS MARKERS FOR MEASURING OVERALL APPARENT DIGESTIBILITY OF SOME DIETARY NUTRIENTS FOR PIGS

A.W. Jongbloed[1], J.G.M. Bakker[1], P.W. Goedhart[2], F. Krol-Kramer[1]

1 Research Institute for Animal Feeding and Nutrition, Lelystad, the Netherlands
2 Agricultural Mathematics Group, Wageningen, The Netherlands

Abstract

Apparent overall digestibility of dietary nutrients is often measured with marker methods. In this study three markers are compared with respect to precision. The used markers are chromic oxide in the usual concentration of 10 g kg^{-1}, HCL-insoluble ash and chromic oxide in a much lower concentration of 0.5 g kg^{-1}. Before Cr_2O_3 was added to the diet it was mixed with maize starch in the proportion 1:3 (w/w) and milled through a 0.5 mm sieve.

For feeds it was found that the coefficient of variation of both sampling and chemical analysis was about one half for the 0.5 g Cr_2O_3 kg^{-1} marker as compared with the 10 g kg^{-1} marker. For faeces the coefficient of variation of chemical analysis was also halved. The coefficient of variation of chemical analysis in feeds of the HCL-insoluble ash marker was twice as large as compared with the 0.5 g Cr_2O_3 kg^{-1} marker, but lower in faeces.

The diluted Cr_2O_3 marker was also compared with the HCL-insoluble ash marker, both with and without addition of Diamol*, with respect to variation between pens and between days within pens of digestibility for dry matter, nitrogen, calcium and phosphorus. The Cr_2O_3 marker resulted in lower variances of digestibility, particularly for the minerals. Addition of Diamol to the HCL-insoluble ash method gave also lower variances.

Keywords: pigs, digestibility, variance, chromium oxide, HCL-insoluble ash

Introduction

Apparent overall and ileal digestibility of dietary nutrients for pigs are often measured using a marker method (Moore, 1957; Daccord, 1983; Yen et al., 1983). Among many markers chromic oxide and HCL-insoluble ash are regarded as most useful and reliable. From the literature it can be concluded that chromic oxide is added in a concentration varying from 5 to 10 g kg^{-1} diet. Such high concentrations have several disadvantages such as the relatively high price of Cr_2O_3, its threat to the environment, its possibly negative effect on palatability and smell of the diet (particularly when soaked for 12-24 hours), its possibly toxic effect on animals and its influence on utilization of some minerals. To overcome these disadvantages, we evaluated Cr_2O_3 as a marker in a much lower concentration of 0.5 g kg^{-1} diet.

* Diamol®: a fine mixture of diatoms

Another objective was to compare the apparent overall digestibility of the nutrients dry matter, nitrogen, calcium and phosphorus using HCL-insoluble ash (with or without 10 g Diamol kg^{-1}) versus Cr_2O_3 in a concentration of 0.5 g kg^{-1}. Also components of variance related to the sampling system were estimated and compared.

Materials and Methods

Firstly data obtained in studies of Oude Elferink et al. (1987), Everts and Smits (1987) and Jongbloed (1987) were statistically re-analyzed. They used as markers HCL-insoluble ash (without Diamol) and/or Cr_2O_3 in concentrations of 10 or 0.5 g kg^{-1}. Prior to addition of Cr_2O_3 it was mixed with maize starch in the proportion of 1:3 (w/w) and milled through a 0.5 mm sieve. It was then mixed thoroughly with the other ingredients and then the diet was pelleted. Several feeds were sampled on different occasions and each sample was analyzed in simple or duplicate. Faeces were always sampled in simple; each sample of feed was analyzed in simple, duplicate or triplicate. So for feeds between sample variation and between duplo within sample variation can be estimated, while for faeces only the latter can be estimated. The number of samples of diets and faeces, analyzed in simple or duplicate/triplicate, for each marker is given in Table 1.

Table 1. Number of samples of feeds and faeces analyzed in simple or duplicate

Marker in feed	Number of feeds and faeces	Number of samples	Numbers analysed simple	duplicate or triplicate
0.5 g Cr_2O_3/kg	feeds 16	49	24	25
	faeces 36	36	-	36
10 g Cr_2O_3/kg	feeds 2	12	8	4
	faeces 13	33	-	13
10 g Diamol/kg	feeds 2	6	-	6
HCl-insoluble ash	faeces 36	36	-	36

Roughly half of the feeds was analyzed in simple, the other half in duplicate. All faeces samples were analyzed in duplicate or triplicate.

Secondly an experiment was setup to compare HCL-insoluble ash, with and without Diamol, with Cr_2O_3 in a concentration of 0.5 g kg^{-1}. Two diets were composed. A basal feed was composed of the following feedstuffs: barley 100 g kg^{-1}, maize 200 g kg^{-1}, tapioca 355 g kg^{-1}, soybean meal 220 g kg^{-1}, alfalfa 48 g kg^{-1}, cane molasses 40 g kg^{-1}, animal fat 20 g kg^{-1} and the remainder was vitamins and minerals. The basal feed was used to estimate digestibility with HCL-insoluble ash, and furthermore the basal feed was supplemented with 0.5 g kg^{-1} Cr_2O_3 and with 10 g Diamol kg^{-1}. The digestibility of the second feed was estimated by both Cr_2O_3 and by HCL-insoluble ash. The feeds were fed to 6 pens, with 4 animals per pen which were fed individually. The feed was offered twice a day according to a feeding scale. Faeces were collected during 3 weeks at about 40, 64 and 90 kg live weight, respectively. In each week at 3 days fresh faeces were collected in the morning and in the afternoon, starting one hour after feeding. Feed samples were taken every week. Feed and faeces were analyzed for dry matter, ash, nitrogen, calcium, phosphorus and HCL-insoluble ash.

Variance components were estimated by analysis of variance (Snedecor and Cochran, 1980).

Results and Discussion

Variation coefficient of sampling and chemical analysis

For the data obtained in the studies of Oude Elferink et al. (1987), Everts and Smits (1987) and Jongbloed (1987) variances between samples (σ^2 sample) and between analyses within samples (σ^2 analysis) were estimated. The estimate between samples variance of HCL-insoluble ash in feeds was negative due to the small number of samples. The estimate was rounded to zero. The mean concentration, variances and variation coefficients are presented in Table 2.

Table 2. Mean concentration of Cr and HCL-insoluble ash (g kg^{-1} dry matter), variances between samples (σ^2 sample) and between analyses within samples (σ^2 analysis) and variation coefficients

	Feed			Faeces		
	Cr diluted	Cr not diluted	HCl-ash	Cr diluted	Cr not diluted	HCl-ash
mean concentration	0.37	6.73	22.19	1.84	20.62	141.3
σ^2 sample	0.000047	0.0529	0.0	-	-	-
CV	0.018	0.034	0.0			
σ^2 analysis	0.000048	0.0603	0.740	0.000542	0.279	1.18
CV	0.019	0.036	0.039	0.013	0.026	0.008

It is seen that the variation coefficient of analysis of the 0.5 g kg^{-1} Cr_2O_3 marker is about one half of that of the 10 g kg^{-1} marker, both in feed and in faeces. The HCL-insoluble ash marker is comparable with the high concentration Cr_2O_3 marker for feeds, but has a smaller coefficient of variation for faeces.
For feeds the variance of the mean of n samples each analyzed in k-fold equals σ^2mean = σ^2sample/n + σ^2analysis/nk. It is thus more profitable to enhance the number of samples than the number of analysis in each sample. For faeces variation among animals or pens should also be taken into account.

Comparison between Cr_2O_3 and HCL-insoluble ash as markers

For the data obtained in the experiment, variances between pens and between days within pens were separately estimated for each period and sampling time within day (morning or afternoon). The variances were not systematically different in the 3 periods and so they were averaged over periods. The averages are given in Table 3.

Table 3. Between pens and between days within pens variances, averaged over periods, of digestibility of nutrients per part of day

Component	Method*	day		pen	
		morning	afternoon	morning	afternoon
Dry matter	1	4.26	4.15	0.96	0.71
	2	2.10	3.25	1.51	0.88
	3	0.82	0.53	1.27	0.49
Nitrogen	1	16.58	16.16	7.12	6.01
	2	9.09	13.72	4.53	3.89
	3	3.19	3.28	3.56	1.68
Calcium	1	44.03	45.66	6.05	0.41
	2	22.09	25.36	8.99	9.51
	3	7.19	3.70	6.47	2.64
Phosphorus	1	83.10	92.13	17.40	5.45
	2	36.35	56.34	9.92	11.88
	3	8.96	9.53	4.76	3.43

*
1 = HCl insoluble ash
2 = HCl insoluble ash with Diamol
3 = Cr_2O_3 (0.5 g kg^{-1})

The variances in the morning and afternoon are similar. The between day within pen variance is much larger than the between pen variance. The Cr_2O_3 marker gave much smaller variances than the HCL-insoluble ash markers, although addition of Diamol also lowered the between day variance.
With p pens and each pen sampled for d days, the variance of the mean digestibility equals σ^2mean = σ^2pen/p + σ^2day/pd. Based on the values in Table 3, σ^2mean calculated with p=6 and d=3 is given in Table 4.

Table 4. Variance of mean digestibility of 6 pens, each pen samples for 3 days in one week

method*	1	2	3
dry matter	0.37	0.35	0.18
N	2.00	1.34	0.62
Ca	2.96	2.86	1.06
P	6.77	4.39	1.20

* see Table 3

From Table 4 it can be concluded that the variance for all components, but particularly for the minerals, is lowest with Cr_2O_3 as marker. The variance of the HCL-insoluble ash method supplemented with Diamol are in favour of those without Diamol. These results might be influenced by an interaction between Cr_2O_3 and HCL-insoluble ash with Diamol which were both added to the basal feed.

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EFFECT OF THE AMOUNT AND COMPOSITION OF THE DIET ON GALACTOSAMINE FLOW FROM THE SMALL INTESTINE

M. F. Fuller & A. Cadenhead
Rowett Research Institute
Aberdeen AB2 9SB, U.K.

Abstract

Of all the nitrogenous substances secreted into the digestive tract mucins may make the greatest contribution to amino acid loss from the ileum because of their resistance to digestion and recycling. The flow of galactosamine at the terminal ileum was estimated in growing pigs given diets, including protein-free diets, at different rates, with or without added fibre. The ratio of galactosamine:N varied with both the amount and composition of the diet.

Introduction

There are no ready means of distinguishing between the endogenous and the exogenous components of the digesta. Because of their resistance to proteolysis the mucins secreted by the goblet cells and other glycoconjugates secreted by enterocytes may represent one of the most important contributions to the flow of unreabsorbed endogenous N out of the small intestine. The contribution of these glycoconjugates may be estimated from the flow of galactosamine, provided that galactosamine forms a constant fraction of these secretions and provided also that the diet does not contain any of the animal products that themselves contain galactosamine. The aim of these experiments was to see how the flow of galactosamine past the terminal ileum was affected by the amount and composition of the diet.

Materials and Methods

Pigs of approximately 80 kg were used. They were fitted with T-cannulas at the terminal ileum. In the first experiment they were given one of three diets in sequence. Diet 1 was protein-free (N 0.27 g/kg), containing (g/kg) maize starch 360, glucose 157 and sucrose 386. Diet 2 was the same as diet 1 but contained casein (71g/kg) and amino acids at the expense of maize starch and was designed to simulate an 'ideal' protein (N 14.4 g/kg). The third diet was based on barley (445g/kg) and wheat (466g/kg) with a total N content of 15.2 g/kg. All three diets were given at a rate of 2.0kg/d. in two equal meals.

In the second experiment there were two diets, the protein-free diet of experiment 1 and this diet with wheat bran (150g/kg) added at the expense of maize starch. These diets were given at rates of either 1 or 3 kg per day.

In both experiments chromic oxide used as a marker in all diets. After the pigs had been given the diets for 6 days digesta were collected continuously for 8h on the 7th day.

Samples of freeze-dried digesta (ca 50mg) were hydrolysed with 2.5ml 4M HCl for 4h at 100° in sealed tubes under vacuum then diluted to 10ml with distilled water. The concentrations of amino sugars were then estimated by ion-exchange chromatography.

Results

Experiment 1.

The results are shown in Table 1. The apparent digestibility of N to the terminal ileum was 0.85 for the casein diet and 0.72 for the cereal diet (SEM 0.0196). The highest daily flow of galactosamine was observed with the protein-free diet; this was significantly reduced by addition of protein, whether from cereals or from casein and amino acids. The mean rates of galactosamine flow with these three diets were 2.0, 1.0 and 1.1 g/d (SEM 0.29) and the corresponding ratios of galactosamine:N (g/g) were 0.33, 0.12 and 0.28 (SEM 0.026).

Table 1. Experiment 1. rates of flow (g/d) at the terminal ileum of dry matter, nitrogen and galactosamine in pigs given a protein-free diet (PF), the same diet with casein and amino acids added (CAA) and a diet based on a mixture of barley and wheat (BW).

Diet	PF	CAA	BW	SEM
Dry matter	377	276	508	37
Nitrogen	5.8	4.3	9.1	1.24
Galactosamine	2.04	1.14	0.97	0.29
ratio galactosamine: nitrogen	0.33	0.28	0.12	0.0273

Experiment 2.

The results are shown in Table 2.

Table 2. Experiment 2. Rates of flow (g/d) at the terminal ileum of dry matter, nitrogen and galactosamine in pigs given a protein-free diet (PF) at rates of 1 or 3kg/d. or the same diet with added wheat bran (PFB) also at rates of 1 or 3 kg/d.

Diet	PF		PFB		
Intake (kg/d)	1	3	1	3	SED
Dry matter	107	265	148	853	58.8
Nirogen	3.6	7.3	3.1	14.7	1.46
Galactosamine	1.48	1.93	0.80	4.13	0.382
ratio galactosamine: nitrogen	0.44	0.28	0.26	0.29	0.039

Discussion

The same protein-free diet was given in both experiments. In the first, when the daily intake was 2kg, the ileal N flow was 5.8g/d, intermediate between the values of 3.6 and 7.3g/d measured in the second experiment with intakes of 1 and 3kg/d., an average increase of approximately 1.7gN per 1kg increase in daily dry matter intake. Galactosamine flow, however was higher in the first experiment than in the second, although not significantly. It is of interest that the ileal nitrogen flow was reduced by the addition of casein and amino acids to the protein-free diet, giving an estimate of the true digestibility of nitrogen of greater than 1.0. In other experiments with these same diets (Wang & Fuller, 1989) the rates of flow were much more closely similar. The addition of casein and amino acids also resulted in an approximately proportional reduction in the flow of galactosamine, the ratio of galactosamine: total N in the digesta remaining approximately the same. In contrast, the substantial increase in ileal nitrogen flow with the cereal diet was accompanied by the lowest galactosamine flow of any amongst these three diets, with a substantial and significant change in the ratio of galatosamine: nitrogen.

With the threefold increase in intake of the protein-free diet (experiment 2) the flow of N past the terminal ileum doubled from 3.6 to 7.3 g ($p<0.05$) and the galactosamine flow increased from 1.5 to 1.9 g (N.S.); the ratio of galactosamine:N fell from 0.44 to 0.28 ($P<0.05$). When the intake of the protein-free diet with added wheat bran was increased from 1 to 3 kg/d the nitrogen flow at the terminal ileum increased over fourfold, from 3.1 to 14.7 g/d with an approximately proportionate increase in the rate of galactosamine flow from 0.80 to 4.1 g ($P<0.05$); in this case the ratio of galactosamine:N was little changed (0.26 to 0.29; NS).

There are several reasons why the ratio of galactosamine: nitrogen might change with both the amount and composition of the diet. First, mucins from different parts of the digestive tract vary in their contents of particular amino sugars and it may be that mucin secretion is not stimulated to the same extent in different parts of the gastrointestinal tract. However, the galactosamine content of porcine small-intestinal mucus (189mg/g glycoprotein; Mantle & Allen, 1981) and submaxillary gland mucus (215mg/g; Katzman & Eylar, 1966) appear rather similar in this respect. Second, although our hypothesis is that mucins form a major fraction of the unrecycled endogenous secretions leaving the ileum, there are undoubtedly other components which may also be stimulated to a greater or lesser extent than the mucins by the nutritional stimuli used here. These other components of the flow of nitrogenous materials include bacterial protein. It is now recognised that there can be substantial microbial activity proximal to the caecum (Jensen *et al.*, 1987; Jensen, 1988) and that microbial protein will make a commensurate contribution to nitrogen flow from the ileum. No measurements were made in these experiments to estimate the bacterial contribution to ileal digesta but it is possible that the increased amount of fibre provided by 3kg of the diet with added bran allowed enhanced microbial activity in the distal ileum, giving rise to the observed increase in nitrogen flow.

As regards our original objective - to examine the constancy of the contribution of galactosamine to ileal nitrogen flow - the results suggest that the ratio of galactosamine:N in the endogenous secretions leaving the ileum varies with both the amount and composition of the diet. Further work is required to identify other specific components of ileal digesta and the way in which they are affected by nutritional conditions.

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PRELIMINARY EVALUATION OF A NEW CANNULATION TECHNIQUE (STEERED ILEO-CAECAL VALVE) FOR QUANTITATIVE COLLECTION OF DIGESTA FROM THE SMALL INTESTINE OF PIGS

Z. Mroz, A.W. Jongbloed, P.A. Kemme, H. Everts, A.M. van Vuuren and R. Hoste

Research Institute for Livestock Feeding and Nutrition (I.V.V.O.), Lelystad

Abstract

Preliminary experiment was carried out on seven barrows (YxFLxDL) of 40-50 kg live weight, fitted with either the post-valvular T-caecum (PVTC) cannulae or the steered ileo-caecal valve (SICV) cannulae and fed three diets, containing recommended (SD), low (LP) and high (HP) levels of protein.

The SICV cannulation is a new technique for quantitative collection of ileal digesta, and in principal it approaches the same anatomical site of the gastro-intestinal tract as the PVTC technique. However, contrary to the latter, caecotomy is omitted, because the valve can be steered into the T-shaped cannula by a system basing on two rings, of which one is introduced round the terminal ileum and another (proximal the first ring in the lumen of the distal ileum.

Ileal apparent digestibility of dry matter, organic matter and nitrogen determined by the SICV technique was markedly lower than when estimated by the PVTC technique, irrespective of diet.

Overall apparent digestibility of these nutrients was similar for both techniques. At the low level of protein supply (diet LP) the values obtained by the SICV technique were slightly higher.

Post-slaughter examination of the gastro-intestinal tract of the pigs (13-14 weeks after surgery) showed anatomo-pathological changes related to proliferation of fibrous tissues between the two rings, dillatation of the distal ileum (muscular hypertrophy 50-100 cm anterior to the ileo-caecal junction) or the development of a collateral passage of digesta. It seems, that this technique may be perhaps used in simultaneous physiological studies of digestion and absorption kinetics, where the time of application is not exceeding 6-8 weeks.

Introduction

Various techniques applied until now for the quantitative collection of ileal digesta from pigs such as re-entrant cannulation, ileo-colic post valve fistulation or ileo-rectal anastomosis, are markedly altering the motility or normal functioning of the gastro-intestinal tract (Laplace and Darcy, 1980; Low, 1980; Köhler et al., 1990). The simple T-shape cannulae fitted at the distal ileum affect far less the physiology of the alimentary tract, but samples of digesta obtained with this technique are regarded to be not representative. This may be due to difficulties arising with even dispersion of a marker in some diets and its fractionation along the stomach and the small intestine caused by differences in specific gravity, reflecting on a different passage time of nutrients and marker (Faichney, 1980; Graham and Åman, 1986).

Recently, van Leeuwen et al. (1988) described the post-valvular T-caecum cannulation (PVTC) which is supposed to enable nearly quantitative collection of digesta from pigs. In this technique, the caecum is removed and replaced by a large T-shaped cannula. However, caecotomy may alter nitrogen digestibility (Gargallo and Zimmermann, 1981). Besides, using this technique there is always some uncertainty that collected digesta may be contaminated with

colonic digesta, particularly at the later stage of growth of the pig, when the gastro-intestinal tract is larger and the ileo-caecal valve does not protrude into the cannula.

Therefore, to eliminate those disadvantages of the PVTC technique and to eliminate using markers for the ileal digestibility measurements, a new quantitative method of digesta collection was developed at the IVVO Lelystad, which is named "the steered ileo-caecal valve" (SICV).

In this preliminary study the apparent ileal and overall digestibilities of dry matter, organic matter, ash, nitrogen and crude fibre from three experimental diets, containing recommended (standard), low and high levels of protein were evaluated using pigs fitted with either the PVTC cannulae or the SICV cannulae.

Materials and Methods

Seven barrows of (Yorkshire x Finnish Landrace) x Dutch Landrace weighing 40-50 kg at the beginning of this experiment were used. Four animals were fitted with the PVTC cannulae according to van Leeuwen et al.(1988), whereas the others were submitted to the SICV technique. After a 14-day recovery period, PVTC cannulated pigs were fed four diets, according to a 4x4 Latin square design. Three of these diets were also given to SICV cannulated pigs and tested according to a 3 x 3 Latin square design.

Main ingredients, composition and nutritive value of the experimental diets are presented in Table 1.

Table 1. Main ingredients, composition and nutritive value of the diets.

Diet	Standard (SD)	Low protein (LP)	High protein (HP)
Main ingredients ($g \cdot kg^{-1}$)			
Maize	-	979	729
Potato protein dried	-	-	250
Barley	315	-	-
Tapioca meal	205	-	-
Wheat	100	-	-
Soybean meal extracted	100	-	-
Peas	55	-	-
Wheat middlings	50	-	-
Fish meal	48	-	-
Analysed composition ($g \cdot kg^{-1}$) diet as fed)			
Dry matter	875	866	872
Organic matter	806	831	838
Crude protein	170	87	244
Ash	69	35	34
Crude fibre	34	16	13
Nutritive value			
ME ($MJ \cdot kg^{-1}$)	13.3	13.6	13.9

The diets were formulated to contain different levels of crude protein, i.e. about 170 $g \cdot kg^{-1}$ for the standard diet (SD), 90 $g \cdot kg^{-1}$ for the low protein diet (LP) and 240 $g \cdot kg^{-1}$ for the high protein diet (HP). Metabolizable

energy content was assumed to be as similar as possible.

The pigs were kept in metabolic cages and offered the diets twice daily in a wet, mash form on a feeding level equivalent to 2.3 times maintenance requirement (maintenance equals 418 kJ·ME $W^{0.75}$).

Experimental periods lasted 25 days in which 10 days of the adaptation period. In each of the 15-day measuring periods, faeces were collected quantitatively for 10 days and thereafter on the 11-th and the 15-th day ileal digesta was also collected quantitatively for 24 hours. Representative samples of diets, digesta and faeces were analysed for dry matter, ash, nitrogen and crude fibre according to the standard procedure. Apparent ileal and overall digestibilities of these nutrients were calculated by the direct method.

Principal lay out of the SICV technique

The SICV cannulation approaches in principal the same anatomical site of the gastro-intestinal tract as the PVTC technique, i.e. the change of ileum into the caecum-colon, where the ileo-caecal valve is protruded slightly into the caecum. Animals were anaesthesized through inhalation anaesthesia, the flank area was shaved and desinfected. A paracostal laparotomy (about 10 cm above mammary tissue) was used to gain entrance to the peritoneal cavity. After locating the ileo-colic valve, a 2-3 cm long transsection of the caecal wall across the ileo-colic valve was made and a 2.4 cm in diameter inner metal ring (with silicon layer) combined with a thread (30 cm long) was introduced into the distal ileum for about 10 cm through the valve. From outside of the ileo-colic junction an outer ring (made of PVC covered with silicon) was fitted around the terminal ileum, close to the caecal wall. This outer ring had a slightly smaller diameter than the inner one, which allowed to maintain the latter permanently inside of the terminal ileum. Afterwards, the thread bound to the inner ring was carried out through a silicon T-shaped cannula, of which the barrel was subsequently placed through the caecal opening, directly across to the ileo-caecal valve and the gut wall was sutured using a double purse-string suturing. Collection of ileal digesta was done by pulling the thread so that the ileo-colic valve could be steered inside of the T-cannula, whereas the outer ring automatically blocked the opening to the caecal-colonic as it is presented in Fig 1.

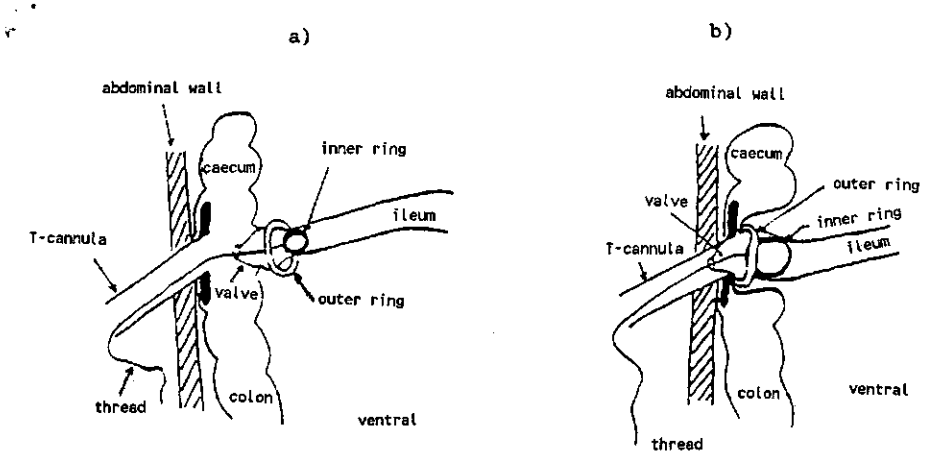

Fig. 1. A schematic view of the SICV technique: a) normal situation when digesta is not collected; b) situation when digesta is collected.

Results and Discussion

Performance of the pigs fitted with either the PVTC or the SICV cannulae depending on the dietary treatments is presented in Table 2.

Table 2. Comparison of growth and feed conversion ratios of pigs fitted with the PVTC and the SICV cannulae depending on the dietary treatments.

Cannulation technique	PVTC		SICV	
	mean	STD*	mean	STD
Daily gains (g)				
standard diet (SD)	895	207	917	226
low protein diet (LP)	315	113	483	105
high protein diet (HP)	681	145	1069	145
Feed conversion ratio				
standard diet (SD)	1.77		1.71	
low protein diet (LP)	5.24		3.52	
high protein diet (HP)	2.21		1.45	

* STD - standard deviation

Protein supply in the diets reflected directly on the daily gains of the pigs. When the standard diet was fed their growth and feed conversion ratios were very similar. More substantial differences in favour of the SICV cannulated pigs were monitored for the other diets.

The apparent ileal digestibility of dry matter, organic matter, ash (for the standard diet only) and nitrogen is given in Table 3.

Table 3. Comparison of the ileal apparent digestibility (%) of dry matter, organic matter, ash and nitrogen in pigs fitted with the PVTC and the SICV cannulae, depending on the dietary treatments.

Cannulation technique	PVTC		SICV	
	mean	STD	mean	STD
Standard diet (SD)				
dry matter	76.3	4.8	73.3	6.6
organic matter	78.6	4.3	76.2	6.1
ash	46.1	11.0	36.9	12.4
nitrogen	82.0	3.1	77.4	5.1
Low protein diet (LP)				
dry matter	82.2	3.7	76.2	2.6
organic matter	82.6	3.3	80.2	2.5
ash	59.6	28.2	-	-
nitrogen	74.6	6.1	62.2	7.6
High protein diet (HP)				
dry matter	78.9	1.8	76.4	2.9
organic matter	80.9	1.8	79.7	2.4
ash	31.7	5.7	-	-
nitrogen	82.8	2.2	74.8	5.2

Values obtained from the SICV cannulated pigs are markedly lower, irrespective of treatment. It indicates that by using the PVTC cannulae ileal digesta is collected incompletely or there were differences in the passage rate.

The overall digestibility of dry matter, organic matter, nitrogen and crude fibre was very similar for both techniques, and at the low level of protein supply (diet LP) the values obtained for the SICV technique were slightly higher (Table 4).

Table 4. Comparison of the overall apparent digestibility (%) of dry matter, organic matter, nitrogen and crude fibre in pigs fitted with the PVTC and the SICV cannulae, depending on the dietary treatments.

Cannulation technique	PVTC		SICV	
	mean	STD	mean	STD
Standard diet (SD)				
dry matter	85.2	1.3	84.5	1.3
organic matter	87.7	1.2	87.2	1.0
nitrogen	85.0	1.6	84.6	2.0
crude fibre	41.2	10.0	36.2	6.9
Low protein diet (LP)				
dry matter	88.8	1.4	89.2	0.4
organic matter	90.1	1.3	91.0	0.5
nitrogen	80.0	4.3	81.7	0.5
crude fibre	38.7	8.2	-	-
High protein diet (HP)				
dry matter	89.5	0.6	89.2	0.4
organic matter	90.7	0.5	90.9	0.3
nitrogen	91.4	0.4	90.9	0.1
crude fibre	43.0	4.7	-	-

Post-slaughter examination of the gastrointestinal tract of the pigs at the end of the experiment (13-14 weeks after surgery) showed anatomo-pathological changes related to proliferation of fibrous tissues between the two rings, dilatation of the distal ileum (muscular hypertrophy from 50-100 cm anterior to the ileo-caecal junction) and in one case a small collateral passage of digesta was found. It seems, that this technique may be still improved and perhaps used in simultaneous physiological studies of digestion and absorption kinetics, where the time of application is not exceeding 6-8 weeks.

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^{51}CR-EDTA IS A DIGESTIVE MARKER MORE ACCURATE THAN ^{14}C-PEG 4000 FOR MEASUREMENT OF NET WATER ABSORPTION FROM PIG'S PROXIMAL COLON

V. Théodorou, J. Fioramonti and L. Buéno

Department of Pharmacology, I.N.R.A., BP 3, 31931Toulouse , France

Abstract

Both polyethyleneglycol (molecular weight 4000, PEG 4000) and ^{51}Cr-EDTA are currently used water soluble and unabsorbed digestive markers. For an easier dosage, PEG is also used in its radioactive form ^{14}C-PEG. The aim of this study was to determine a digestive marker accurate for determination of colonic net water absorption in conscious pigs. For that we have compared values of water absorption using ^{14}C-PEG 4000 and ^{51}Cr-EDTA.
Key words : ^{14}C-PEG 4000, ^{51}Cr-EDTA, helicoidal colon, net water absorption, pigs

Introduction

In addition to their use as digestibility indicators the water-soluble markers as PEG 4000 and Cr-EDTA have been useful in study of water balance in man and animals (Whalen et al., 1966 ; Ishikawa, 1965 ; Jacobson et al., 1963 ; Hecker, 1971).

In 1953 Sperber et al. reported the use of PEG as a reference substance in studies of ruminant digestion and found that it was neither absorbed nor destroyed to any considerable extend in the digestive tract and more than 90 % was recovered in the feces. Indeed PEG compounds are actually used in high enough molecular weight, about 4000, to avoid any absorption or degradation.

Moreover, the lack of a specific sensitive and accurate method for the analysis of PEG has been seen as a serious limitation in its use. The use of radioactive PEG has been proposed to overcame this difficulty.

The complex of ^{51}Cr with ethylenediamine tetraacetic acid (^{51}Cr-EDTA) has been suggested by Downes and McDonald (1964) as a substitute for PEG. ^{51}Cr-EDTA has been found to bind to a small extend to particulate matter in the rumen of sheep and to be absorbed and subsequent excreted in the urine at small but not significant percentages always less than 5 % and mostly around 3 % in sheep or less in cattle. Feces recovery has been found good ($\simeq$ 95 %) (Downes and Mc Donald, 1964). The aim of this study was to determine a water soluble digestive marker accurate for determination of colonic net water absorption in conscious pigs.

Materials and Methods

Animal preparation

This study was carried out using four Large White pigs, each weighing approximately 40 kg at the beginning of the experiments. The animals were housed in individual cages. They were on a standard diet for growing pigs (30

g/kg body weight per day) of concentrates of barley, wheating and fish meal (Rental, 31770 Colomiers,) given in two daily meals, and had free access to water.

Under thiopental anaesthesia, each animal was fitted with a silicone catheter into the lumen of the terminal ileum (at 20 cm from the ileo-caecal junction) and two silicone cannulas (internal diameter 8 mm) were inserted in the helicoidal (proximal) colon. The proximal cannula was placed on the first colonic coil and the distal cannula, at about 50 cm from the first one, on the second coil. The two cannulas and the catheters were exteriorized on the left flank. The animals were allowed to recover for 2 weeks before beginning the experiments.

Net water flux measurement

Saline containing either ^{14}C-PEG 4000 or ^{51}Cr-EDTA at a concentration of approximately 0.1 μCi/ml was continously infused 24 h/day at a constant rate of 20 ml/h through the ileal catheter. Determination of ^{14}C-PEG 4000 and/or ^{51}Cr-EDTA in colonic samples (about 2 g) taken through the two cannulas and in the perfused solution was performed by liquid scintillation (Intertechnique SL20 scintillation spectrometer, Plaisir, France) and γ-counter (MG 252, Kontron, Basel, Switzerland) respectively.

Dry matter of colonic samples was determined by heating about 1 g at 100°C for 24 h. Net water flux in the colonic segment situated between the two cannulas corresponded to the difference between the flow of the liquid phase of digesta at the level of oral cannula (f_1) and the flow of the liquid phase at the level of the aboral cannula (f_2). Flows were calculated as follows :

$$f_1 = \frac{F_o[C_o - (C_1/q_1)]}{C_1/q_1} \quad \text{and}$$

$$f_2 = \frac{F_o[C_o - (C_2/q_2)]}{C_2/q_2}$$

where F_o is the rate of marker infusion, C_o is the concentration of digestive marker in the perfused solution, C_1 and C_2 are the concentrations in the samples taken from the oral (C_1) and the aboral (C_2) cannulas and q_1 and q_2 are the percentages of water in the samples taken from the oral (q_1) and the aboral (q_2) cannulas.

In two supplementary pigs a ^{51}Cr-EDTA solution (0.1 μCi/ml) was continuously infused (20 ml/h) into the ileum for 24 h and a blood sample (10 ml) was taken from the marginal vein of the ear just before the infusion was stopped. No radioactivity was detectable in the blood of each pig.

Experimental schedule

For determining the colonic net water absorption, samples of colonic contents (2 g) were taken through the two cannulas at 2 h intervals for 10 h in each animal. The first sample was obtained 2 h after the morning meal given at 8.00 h.

All experiments were repeated twice in each pig. Statistical analysis of the results was performed using arithmetic means ± standard deviation of mesurement and Student's paired t test.

Results

Values of net water flux obtained using ^{14}C-PEG 4000 indicated a net secretion of water into the colonic lumen during the 2nd, 4th and 6th hour after the morning meal (-2.7 ± 0.2 ; -1.9 ± 0.4 and -0.9 ± 0.2 respectively) (fig. 1). This secretion disagrees with colonic contents dry matter percentage significantly ($P < 0.05$) higher at the distal cannula than the proximal (40.2 ± 0.9 and 35.3 ± 2.4 respectively on the 2nd postprandial hour ; table 1). On the contrary, the use of ^{51}Cr-EDTA, permitted to quantify colonic water rates absorbed ; which were increasing from 0.7 ± 0.1 ml/min on the second postprandial hour to 1.7 ± 0.3 ml/min on the eight hour (Fig. 1).

Table 1.

	Colonic contents dry matter (%) ^{14}C-PEG 4000	
Posptrandial hours	Proximal cannula	Distal cannula
2	35.3 ± 2.4	40.2 ± 0.9*
4	34.9 ± 1.6	41.3 ± 0.6*
6	37.1 ± 0.6	43.4 ± 2.1*
8	39.2 ± 1.3	45.3 ± 2.1*
10	38.3 ± 1.7	43.4 ± 1.8*

*significantly different ($P < 0.05$ from proximal cannula dry matter values.

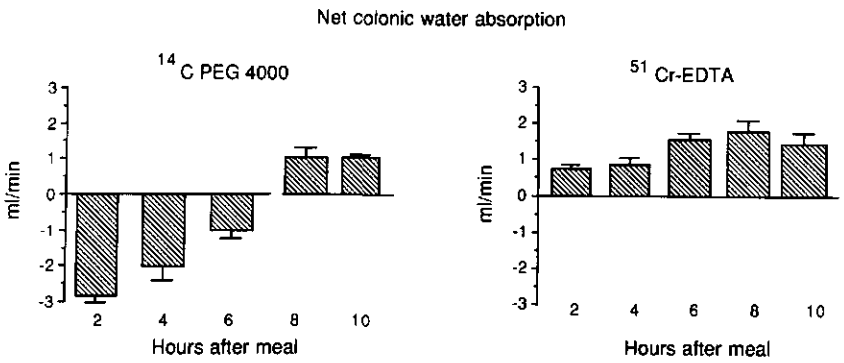

Fig. 1. Postprandial pattern of net colonic water absorption (means ± SD, n = 8) ^{14}C-PEG 4000 led to erroneous values indicating a net water secretion into the colonic lumen, in basal conditions. Assays with ^{51}Cr-EDTA permitted to quantify net colonic water absorption in the proximal colon of pigs.

Discussion

Most of the studies related to colonic water absorption concern absorption from a solution infused into an emptied colon (Phillips, 1987). The technique we used permits the evaluation of water absorption from a normal colonic

content in more physiological conditions. It has been shown in sheep that cannulation of the large intestine does not modify the movements of digesta (Mc Rae et al., 1973) and we can postulate that in our study the presence of two cannulas did not alter colonic functions. On the other hand a rate of infusion of 20 ml/h into the ileum seems to be negligible by comparison with the rate of flow of digesta determined at the level of the first colonic coil.

Moreover the use of ^{51}Cr-EDTA as a water-soluble marker seems suitable for water absorption measurement in the pig colon since no radioactivity has been found in blood after a 24 h infusion into the ileum. Moreover, in sheep, ^{51}Cr-EDTA is known to bind only to a small extend to solid particles and to have a recovery slightly better than that of polyethyleneglycol (Downes and Mc Donald, 1964).

On the contrary use of ^{14}C-PEG led to erroneous values. Indeed recoveries of PEG in feces were frequently somewhat low (Smith, 1958) and a mean loss of about 10 % occurred in the large intestine at calf. Hyden (1950) suggested that the losses were due to some degradation of PEG, as he obtained good recoveries in vitro trials by his method of turbidimetric analysis. The turbidity of polyethyleneglycol decreases with decreasing of its molecular weight. It therefore appears that no good recovery can be obtained when PEG is degraded to the homologues of lower molecular weight. An in vivo study (Ishikawa, 1965) undertaken to make a qualitative test for degraded polymers in feces and ingesta at wine fed PEG, did not show any degradation at this digestive marker through the digestive tract.

In conclusion, ^{51}Cr-EDTA is a digestive marker more accurate than ^{14}C-PEG 4000 for determiantion of net water absorption from pig's proximal colon. A hypothetic explanation for that way be a partial degradation of PEG by colonic microflora with absorption fragments containing ^{14}C which are situated at the extremity of the carbon chain.

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IN VITRO DETERMINATION OF PROTEIN DIGESTIBILITY OF FEEDS USING A "DIGESTION CELL" (PRELIMINARY RESULTS)

D. Thomaneck, U. Hennig and W.-B. Souffrant

Research Centre of Animal Production Dummerstorf-Rostock
Division of Animal Nutrition "Oskar Kellner", Rostock, Germany[1)]

Abstract

The enzymatic digestibility of different proteins was determined using a special digestion cell. The "digestion cell method" is based on a two-step proteolysis with pepsin and pancreatin. The pancreatin hydrolysis is combined with dialysis through a membrane with a defined molecular weight cut off.
The in vitro results were compared with results of the corresponding in vivo experiments.
Key words: digestibility, in vitro, proteolysis, dialysis

Introduction

The substitution of expensive and time-consuming in vivo experiments by simple in vitro determinations is one of the aims in the research of the digestibility of feeds for animals. Recently, Savoie published a relatively simple in vitro method for the determination of protein digestibility (1,2). This method is based on a two-step proteolysis and the N (nitrogen)-determination of the dialysate obtained by dialysis of the enzyme-treated feeds in a specially constructed digestion cell (1). The applicability of this "digestion cell method" was tested using proteins of different sources. Results obtained were compared with corresponding in vivo experiments.

Materials and Methods

Enzymes: Pepsin conc. (5000 ES, VEB Berlin-Chemie)
Pancreatin (4 NF, Biochemie Bernd Belger)

Feeds: The following protein sources were used: casein, maize, peas "Grapis", meat meal (10,9 % crude fat), solvent extracted meals of soybean, linseed (6% crude fat), cottonseed, sunflowerseed and rapeseed (untreated and treated with copper sulfphate to remove inhibitors).

"Digestion cell method". This method and the construction of the cell are well discribed by Savoie et al. and Gauthier et al (1,2). The applied standard procedure consists of the following steps:

1. Present address: Justus-v.-Liebig-Weg 1, Rostock 2500

1. 250 mg protein (40 mg N) are treated with 1 mg pepsin at pH 1,9 and 37 °C for 30 minutes.
2. Following this pretreatment the mixture is calibrated to pH 7,5 by 0,1 N sodium hydroxide and then poured into the inner cylinder of the digestion cell which is surrounded by a dialysis membrane (molecular weight cut off 1000).
3. 10 mg pancreatin is added to the mixture and digestion carried out at 37 °C for 6 hours.
Digested products dialyzed through the membrane into the buffer solution (flow rate 1,6 ml/min) are collected every hour for 6 hours.
4. The N-content (Kjeldahl) and α-amino-N-content using ninhydrine reagent (3) are estimated. The N-digestibility is calculated using the formula:

$$\text{N-digestibility} = \frac{\text{dialyzed nitrogen}}{\text{protein nitrogen (40 mg N)}} \times 100$$

This standard procedure was modified by pepsin digestion for 3 hours. The results were compared with those of the standard procedure. The determination of α-amino-N was carried out to proof the amount of free amino acids in the dialysates in relation to the estimated N-content in the dialysates.

In vivo investigations. The in vivo digestibility of the corresponding feeds was determined as precaecal digestibility by means of end-to-side ileorectal anastomose estimation in pigs (4).

Results and Discussion

The results of the in vitro digestibility of different feeds measured with the standard and the modified procedure are summarized in table 1 in comparison to the in vivo crude protein digestibility by pigs.
Table 2 contains the results of the α-amino-N estimations related to the corresponding N-values.
The general applicability of this method was tested on feeds with known high (casein) and low (meat meal) digestibility. As can be seen in table 1 clear differences were to be found. These results are in agreement with similiar results in literature (2).
Experiments for reproduction of the determination were performed on casein and the results for digestibility of casein (34,2±5,9%, n=7) indicate a sufficient reproducibility.
Investigations concerning the methodology of the standard and modified procedure show clearly that the time of 30 minutes for pre-digestibility is sufficient (table 1). The results of Gauthier et al (2) are confirmed.
The comparison of N-digestibility (table 1) between the different feeds shows no clear differences (casein excluded). The variation between the single values for a selected feed

(maize) ranged from 12,8 to 22,6 % (mean value 17,7; standard deviation 3,6 %).

Table 1. In vitro digestibility (%) of different feeds using the "digestion cell method" in the standard and modified procedure in comparison to the in vivo crude protein digestibility (%) on pigs

feed	Digestibility (%) in vitro standard	in vitro modified	in vivo precaecal
casein	34,2	35,1	87,1*
maize	17,9	17,4	73,6±3,1
peas	15,0	17,6	66,8±8,8
meat meal	21,0	24,9	65,4±2,8
solv.extr.meals of			
soybean	16,2	15,6	79,7±2,7
linseed	19,2	18,0	62,6±5,0
cottonseed	16,7	21,0	71,6±1,4
sunflower seed	20,0	25,4	71,9±3,3
rapeseed			
untreated	21,0	22,6	
treated	15,1	15,8	

* literature (n=10)

Table 2 shows the part of nitrogen in the dialysate in form of α-amino nitrogen. In the mean of all the proofed feeds 55 % of the digested nitrogen were free amino acids. The rest could be oligopeptides. Further experiments are necessary before final conclusions can be made. In this regard it seems necessary, to direct more attention to methodical aspects, especially to the flow conditions in the digestion cell.

Table 2. Results of the α-amino-N-determination, related to the corresponding N-values (%)

feed	% α-amino-N of N, mesasured by standard procedure	modified procedure
casein	64,6	58,2
maize	46,7	55,1
peas	50,0	58,0
meat meal	40,3	37,7
solv. extr. meals of		
soybean	-	71,2
linseed	65,1	54,1
cottonseed	74,9	62,9
sunflowerseed	68,7	59,1
rapeseed		
untreated	61,0	57,0
treated	44,3	38,0

Our preliminary in vitro results were compared with in vivo investigations (table 1) and only a slight relation was found. Correct interpretation of the results is not possible, because of the small number of replications. On the one hand the in vitro results represent more the value for true digestibility (in our experiments for 6 hours pancreatin digestibility) and on the other hand the in vivo results reflect the apparent digestibility.
The aim of further experiments and more replications should be to find regressions between in vitro and in vivo preceacal digestibility values.

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ADAPTATIVE EFFECTS OF ILEO-RECTAL ANASTOMOSIS ON DIGESTION IN PIGS

Torsten Köhler[ab], Rainer Mosenthin[a], Leo A. den Hartog[c], Joop Huisman[d], Martin W.A. Verstegen[b]

[a] Institute of Animal Nutrition and Feed Science, University of Kiel, D-2300 Kiel, FRG.

[b] Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands.

[c] Research Institute for Pig Husbandry, P.O. Box 83, 5240 AB Rosmalen, The Netherlands.

[d] TNO Institute for Animal Nutrition and Physiology (ILOB) P.O. Box 15, 6700 AA Wageningen, The Netherlands.

Abstract

A long term study with 16 barrows (initially weights 30 kg) was carried out to investigate the adaptative effects of ileo-rectal anastomosis (IRA) on digesta composition. Therefore, 9 pigs were provided with an IRA and 7 pigs with a post-valve T-caecum cannula. Digesta were collected 3, 9 and 12 weeks after surgery, respectively. The contents of dry matter, nitrogen, methionine, threonine, leucine, lysine, diaminopimelic acid, volatile fatty acids, sodium and potassium in digesta were measured. Apart from sodium all above parameters were increased ($P < .05$, $P < .001$) in the digesta of IRA pigs (except threonine and lysine in wk 9 after surgery). Sodium concentration in digesta of IRA pigs was distinctly ($P < .001$) lower than in digesta of PVTC pigs. This change of digesta composition in IRA pigs is established already 3 weeks after surgery. This adaptation will therefore have an influence on the digestibility measurement.

Introduction

Ileal digestibility measurement is considered to be the most suitable method to estimate the potential availability of dietary protein for pigs (Zebrowska, 1973). **Ileo-rectal Anastomosis (IRA)** has been proposed as a new technique for measurement of ileal protein and aminio acid digestibilities (Fuller and Livingstone, 1982; Picard et al, 1984; Darcy-Vrillon and Laplace, 1985; Souffrant et al., 1985). IRA was carried out by fitting the distal part of the ileum to the side of the descending colon just before the rectum. Such a bypass of the colon can be

performed as a pre- or post valve (Green et al., 1988) as well as an end-to-end or end-to-side anastomosis. The most important advantage of IRA is that digesta can be easily collected quantitatively via the anus. This is important for diets with a large content of fibrous byproducts. Problems like blockage or leakage which have been reported in re-entrant cannulated pigs (Sauer, 1976; Just et al.1980; Köhler et al, 1990) are avoided. On the other hand, this technique excludes the digestive capacity of the hind gut especially with regard to the absorption of minerals, water and volatile fatty acids (VFA). The objective of the present experiment was to study if there are changes in digestion and absorption of nutrients in pigs fitted with IRA. For comparison pigs fitted with a **Post-Valve T-Caecum (PVTC)** cannula were used.

Materials and Methods

16 crossbred castrates (Yorkshire x Dutch Landrace) (avg. BW 30 kg) were used. Nine pigs were provided with an IRA as described by Köhler et al. (1991a) and seven with a PVTC cannula as described by van Leeuwen et al. (1990). Following surgery the pigs were allowed to recover two weeks. Pigs were housed individually in adjustable cages in an environmentally controlled metabolism unit with continuous light. Air temperature was 19 to 21° C.

TABLE 1. COMPOSITION OF THE EXPERIMENTAL DIET (%)

INGREDIENTS	
MAIZE	32.95
BARLEY	6.00
WHEAT	10.50
SOYBEAN MEAL	22.50
MOLASSES	4.00
POTATO PULP	9.20
BEET PULP	10.00
ANIMAL FAT	1.00
L-LYSINE HCL	0.18
DL-METHIONINE	0.12
PREMIX*	1.00
$Ca(H_2Po_4)*H_2O$	1.50
$CaCO_3$	0.50
NaCl	0.30
Cr_2O_3	0.25

* CONTRIBUTED THE FOLLOWING VITAMIN AND MINERAL SOURCES PER KG OF DIET: RETINOL, 9000 IU; CHOLECALCIFEROL, 1800 IU; α-TOCOPHEROL, 40 mg; MENADIONE, 3 mg; RIBOFLAVIN, 5 mg; COBALAMINE, 40 µg; NICOTINIC ACID, 30 mg; D-PANTOTHENIC ACID, 12 mg; CHOLINE CHLORIDE, 150 mg; ASCORBINE ACID, 50 mg; KJ, 500 µg; $CoSO_4*7H_2O$ 2.5 mg; Na_2SeO_3, 0.2 mg; $FeSO_4*7H_2O$, 0.40 g; $CuSO_4*5H_2O$, 0.1 g; MnO_2, 0.07 g; $ZnSO_4*H_2O$, 0.2 g. THIS MIXTURE ALSO SUPPLIED 20 ppm VIRGINIAMYCIN TO THE DIET.

The composition of the experimental diet is given in Table 1. The pigs were fed at a level of 2.4 times energy required for maintenance (ARC, 1981). Feed was given twice daily at 08.00 and 20.00. Water was administered with the feed at a ratio of 2.5:1. The anastomized pigs also received an extra electrolyte solution (200 ml 20kg LW^{-1} d^{-1}) twice daily as described by Hennig et al.(1986). The live weights of the animals were recorded every two weeks. The ileal digesta were collected quantitatively during 5 d for 12 h/d from 9.00 to 21.00 at 3, 9 and 12 weeks after surgery. Details of the sampling method are described by Köhler et al.(1991a). Chemical analysis of DM, N, AAS, DAPA, VFA, Na and K have been described in

details by Köhler et al. (1991a,b,c). Differences among treatment means were tested according to the GLM procedure using SAS (1985).

Results

Table 2 shows the average concentrations of dry matter, nitrogen and some indispensable amino acids in the digesta of pigs fitted with IRA or PVTC cannulas. Both dry matter and nitrogen concentrations were higher (P< .001) in IRA pigs than in PVTC pigs at 3, 9 and 12 weeks after surgery, respectively. 3 and 12 weeks after surgery the concentrations of MET, TRE, LEU, and LYS were higher (P< .001) in IRA pigs than in PVTC pigs. 9 weeks after surgery, however, these differences were significant for MET (P< .001) and LEU (P< .01) but not for TRE and LYS.

TABLE 2. AVERAGE CONCENTRATION OF DRY MATTER(%), NITROGEN AND SOME INDISPENSABLE AMINO ACIDS (% I.DM) IN DIGESTA OF PIGS FITTED WITH ILEO-RECTAL ANASTOMOSIS (IRA) OR POST-VALVE T-CAECUM (PVTC) CANNULA 3, 9 AND 12 WEEKS AFTER SURGERY. MEANS ±SE

TIME	PERIOD I		PERIOD II		PERIOD III	
	IRA	PVTC	IRA	PVTC	IRA	PVTC
DM	10.17±0.15^{e}	8.44±0.15^{f}	10.67±0.23^{e}	8.85±0.18^{f}	10.86±0.19^{e}	8.79±0.05^{f}
N	3.00±0.05^{e}	2.46±0.08^{f}	2.93±0.07^{e}	2.35±0.06^{f}	2.91±0.06^{e}	2.29±0.04^{f}
MET	0.23±0.01^{e}	0.16±0.01^{f}	0.24±0.01^{e}	0.14±0.01^{f}	0.26±0.01^{e}	0.15±0.01^{f}
TRE	0.76±0.01^{e}	0.65±0.02^{f}	0.71±0.02^{a}	0.66±0.02^{b}	0.72±0.01^{e}	0.61±0.02^{f}
LEU	1.01±0.02^{e}	0.78±0.03^{f}	0.97±0.03^{c}	0.78±0.04^{d}	1.02±0.02^{e}	0.73±0.02^{f}
LYS	0.60±0.01^{e}	0.48±0.02^{f}	0.58±0.02	0.53±0.02	0.78±0.01^{e}	0.58±0.02^{f}

^{ab} MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER P< .01
^{cd} MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER P< .001

Table 3 presents data on the level of different microbial metabolites in digesta collected by the different methods described. In all three periods the amount of DAPA in the digesta of IRA pigs was higher than in PVTC pigs. Moreover, this difference increased continously (P< .05, P< .0001 and P< .0001 from week 3 to 12 after surgery). In all three periods total amount of VFA measured in digesta from IRA pigs was higher (P< .0001) than in PVTC pigs. In PVTC pigs the highest amount of VFA was found three weeks after surgery. In IRA pigs the VFA concentration increased continuously. The acetate / propionate ratios in IRA pigs were different in comparison with PVTC pigs. In IRA pigs this ratio was about 1.80 whereas in PVTC pigs the ratio increased from 3.92 in the 1st period to 6.65 in the 3rd period.

Average concentration of sodium and potassium in digesta of IRA- and PVTC pigs are sammarized in Table 4. In IRA pigs considerable lower (P< .0001) sodium and higher (P< .0001) potassium concentrations were found in all three periods.

Discussion and Conclusions

The measurement of protein and amino acid digestibilities at the distal ileum of pigs has been proposed to eliminate the influence of the bacterial activity in the hind gut on protein digestion (Zebrowska, 1973). However, results given in Table 2 show significant differences between the two methods designed to measure ileal digestibilities. A fundamental difference between both techniques is the passage of digesta through the rectum of the IRA pigs. In a previous paper (Köhler et al. 1991) it was shown that the girth of the rectum in IRA pigs was larger (P< .05) than in

PVTC or intact pigs. As a consequence the volume of the rectum was increased and the digesta flow changed. This may lead to a higher water absorption. Leterme et al.(1990) reported a systematically lower apparent and true N and amino acid digestibility in IRA pigs compared to T-cannulated pigs. This means that the higher concentration of N and amino acids in the digesta of IRA pigs may be the result of an increased secretion of endogenous sources and a lower N and amino acid digestibility.

IRA seems to have an influence on the extent of fermentation. The DAPA measurements in ileal digesta indicate that micribial activity was markedly affected by the type of collection technique. DAPA concentrations in digesta of IRA pigs were higher ($P < .05$ in week 3, $P < .001$ in weeks 9 and 12, respectively) than in PVTC pigs. This indicates a rapid change in bacterial activity between both treatments following surgery. The results for DAPA levels in digesta correspond with the results for volatile fatty acid (VFA) levels in digesta. The amount of VFA in PVTC pigs was comparable with results reported in literature (Clemens et al., 1975; Argenzio & Southworth, 1975; Drochner, 1984; Mosenthin, 1987). In IRA pigs the level of VFA was 3-8 times higher. An influence of the collection technique was also found for the acetate/propionate ratio which was considerable lower ($P < .01$; $P < .001$) in IRA pigs than in PVTC pigs. In PVTC pigs the percentage of acetate was in a range of 74-83 % and in agreement with ileal data given in literature (Argenzio and Southworth, 1974; Clemens et al., 1975; Drochner, 1984; Mosenthin, 1987). In IRA pigs the molare proportion of acetate was about 58 %. This is in agreement with data which have been presented for faeces (Argenzio & Southworth, 1974; Drochner, 1984; Sauer et al., unpublished.).

TABLE 3. AVERAGE CONCENTRATION OF VOLATILE FATTY ACIDS (MMOL/L) AND DAPA (mg/gDM) IN DIGESTA OF PIGS FITTED WITH ILEO-RECTAL ANASTOMOSIS (IRA) OR POST-VALVE T-CAECUM (PVTC) CANNULA 3, 9 AND 12 WEEKS AFTER SURGERY. MEANS AND SE.

TIME	PERIOD I		PERIOD II		PERIOD III	
	IRA	PVTC	IRA	PVTC	IRA	PVTC
DAPA	0.61 ± 0.04^{a}	0.47 ± 0.03^{b}	0.68 ± 0.04^{e}	0.34 ± 0.03^{f}	0.79 ± 0.05^{e}	0.34 ± 0.02^{f}
VFA	112.33 ± 9.20^{e}	31.22 ± 3.71^{f}	116.41 ± 11.48^{e}	21.41 ± 2.86^{f}	162.75 ± 5.79^{e}	20.55 ± 2.60^{f}
C_2:C_3	1.74 ± 0.07^{e}	4.12 ± 0.43^{f}	1.84 ± 0.06^{c}	5.80 ± 1.05^{d}	1.86 ± 0.07^{e}	7.13 ± 0.86^{f}

^{ab} MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER $P < .05$
^{cd} MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER $P < .01$
^{ef} MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER $p < .001$

The average concentrations of sodium and potassium demonstrate the importance of the large intestine for mineral absorption. For sodium a negative ileal digestibility has been reported by different authors (Partridge, 1978; Drochner, 1982; den Hartog et al.,1985; Partridge et al.,1986). A sodium concentration of about 1% i.DM which was found in digesta from IRA pigs compared with 2.8 -3.2% i.DM found in PVTC pigs can be explained as an intestinal adaptation in IRA pigs to conserve sodium. These results are in agreement with results found for the renal sodium excretion which was also extremely lower in IRA pigs ($P < .05$) than in PVTC or intact pigs (Köhler et al. 1991). An increased re-absorption of sodium by the kidneys and an increased sodium absorption at the distal colon (rectum) has been described

TABLE 4. AVERAGE CONCENTRATION OF SODIUM AND POTASSIUM (%i.DM) IN DIGESTA OF PIGS FITTED WITH ILEO-RECTAL ANASTOMOSIS (IRA) OR POST-VALVE T-CAECUM (PVTC) CANNULA 3, 9 AND 12 WEEKS AFTER SURGERY. MEANS AND SE.

TIME	PERIOD I		PERIOD II		PERIOD III	
	IRA	PVTC	IRA	PVTC	IRA	PVTC
Na	1.02±0.04[a]	3.00±0.10[b]	0.97±0.04[a]	2.90±0.07[b]	0.95±0.04[a]	3.24±0.06[b]
K	2.85±0.04[a]	1.29±0.05[b]	2.79±0.06[a]	1.35±0.16[b]	2.86±0.08[a]	1.24±0.09[b]

[ab] MEANS IN THE SAME ROW AND WITHIN A COLLECTION PERIOD WITH DIFFERENT SUPERSCRIPTS DIFFER P< .0001

as a result of an increased aldosterone activity (Rechkemmer, 1990) and is in agreement with a higher weight of the adrenal glands which was found in IRA pigs (Köhler et al. 1991b). In all three periods the potassium concentration in the digesta of IRA pigs was higher (P< .001) than in PVTC pigs. These results are in accordance with results reported by Heitzmann and Drochner (1990). These authors found an increased potassium and decreased sodium concentration in ileal digesta when pigs were fed a diet with a low sodium content.

In conclusion the results of our experiment show a clear adaptation to the missing hind gut digestion in IRA pigs. Moreover, the change in digesta composition could already be observed three weeks after surgery. It can be accepted that this intestinal adaptation has an influence on the digestibility, the intestinal fermentation and the aldosterone activity. Digesta collected in pigs fitted with an end-to-side IRA including the ileo-caecal valve seems to be quite different from ileal digesta collected at the distal ileum in cannulated pigs. Further studies are warrented to investigate the effect of different IRA techniques and different mineral supplementations on digesta composition and digestibility.

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DETERMINATION OF TRYPSIN AND CHYMOTRYPSIN ACTIVITY IN PANCREATIC JUICE, TISSUE AND CHYME: THE EFFECT OF FREEZE DRYING AND STORAGE

M.J. van Baak [1], E.C.Rietveld[1] and C.A.Makkink[2]

[1] TNO-Institute for Animal Nutrition and Physiology (ILOB), Wageningen,

[2] Department of Animal Nutrition, Agricultural University, Wageningen, The Netherlands

Abstract

As a contribution to digestive physiological studies analysis of trypsin and chymotrypsin activities in pancreatic tissue , juice and chyme are carried out. Knowledge of storage conditions of these samples in relation to enzym activity has to be acquired. Several "pilot experiments" were carried out. Pancreatic tissue, juice and chyme were freeze dried and analyzed for trypsin and chymotrypsin activity. Results indicate an improvement of the storage conditions of these biological samples by using the freeze drying technique. For definite conclusions more experiments with more samples will be needed. Experiments are planned in near future.
Keywords: trypsin, chymotrypsin, pancreas, pancreatic juice, chyme, storage conditions, freeze drying.

1. Introduction

Determination of the activity of proteolytic enzymes in different matrices, may have an important contribution in digestive fysiological studies. Fistulation techniques for pancreatic juice collection and chyme collection make it possible to study enzym actvities in these matrices. Usually, the collected samples cannot be analyzed immediately, so they have to be stored. A study on the influence of storage temperature on enzym activities in pancreatic juice has been carried out by Makkink et al (1990). For short or long term storage, freeze-drying might be a possibility to prevent enzyme activity changes. Experiments were carried out to get information about the effect of freeze-drying on the activity of trypsine and chymotrypsine in pancreatic juice, pancreas and chyme.

2. Materials and Methods

2.1 Sample collection

To collect pancreatic juice a castrated male pig, fitted with a pancreatic cannula according to the method of Hee et al (1985) was used. An other pig was fitted with an ileal T-cannula according to the method of van Leeuwen et al (1988) to collect chyme.
By dissection of three piglets and an one year old castrated male pig, homogenized pancreases were obtained. Samples were worked up immediately after collection at the laboratory. Aliquots of each sample were analysed for enzym activities. The remainder of each sample was frozen (-20°C) immediately.

2.2 Freeze drying and storage

The frozen juice, chyme and pancreas were freeze dried the day after sampling. After freeze drying, pancreas and chyme were grounded with a Retsch grinder using a 1mm sieve. The dried pancreatic juice and pancreas were kept in sealed polyethylene containers and stored in a refrigerator. Freeze dried chyme was stored in a sealed container which was placed into a exsiccator filled with silicagel at ambient temperature.

2.3 Reconstitution of the freeze dried material

At 1 gram freeze dried chyme 20 ml demineralized water was added. By means of a Polytron this sample was homogenized. An aliquot of 5 ml was taken and transfered into a tube and was centrifuged during 10 minutes (46000g, 4°C). 50 mg freeze dried pancreatic juice was reconstituted in 2.5 ml demineralized water (4°C). At 200 mg freeze dried pancreas tissue 25 ml demineralized water was added. Homogenization and sub-sampling are carried out as described for chyme.

2.4 Activation procedure

To determine total enzymactivity in pancreatic juice and tissue, zymogens have to be activated. One ml of an enterokinase solution (4.3 U/ml) was added to 1 ml of the reconstituted sample, mixed and placed into an ice-bath. Tissue should be incubated with enterokinase during 24 hours .For the determination of total enzym activity in pancreatic juice an incubation time of 2 hours has to be applied.
Because intestinal mucous membrane contains enterokinase, zymogens will be activated in-vivo. Therefore an incubation of ileumchyme with enterokinase was found to be superfluous.

2.5 Determination of trypsin and chymotrypsin activities (analytical procedure).

Trypsin and chymotrypsin activities were determined according to Bergmeyer.

Table 1. Determination of trypsin and chymotrypsin activities according to Bergmeyer (1974).

	Determination of Trypsin activity	Determination of Chymotrypsin activity
Buffer solution	Tris.HCl buffer solution pH 8.1 ; 2.6 ml	Tris.HCl buffersolution pH 7.8 ; 1.50 ml
Substrate	189.5 TAME / 50 ml bidest ; 300 µl	33.6 mg BTEE / 100 ml methanol/water (1:1, w:w); 1.40 ml
Sample volume	100 µl	100 µl
Wavelength	247 nm	256 nm

2.6 Experiments

Trypsin- and chymotrypsin activities were measured immediately at the day the samples were collected (day 0). The freeze dried samples were analysed at several times. There was no defined chronological table. At times some routine analysis had to be done the freeze dried samples were analysed as well.

3. Results and Discussion

3.1 The effect of freeze drying and storage of pancreatic juice on trypsin and chymotrypsin

In fresh pancreatic juice (at day 0) the trypsin activity was 95.97 U/ml. The activity of chymotrypsin was determined to be 30.01 U/ml. The freeze dried pancreatic juice was analyzed at 4, 68, 70, 75, 82 and 104 days after collection.

Table 2. Trypsin and chymotrypsine activity in pancreatic juice after freeze drying and storing at + 4°C. Activities as a percentage of activity in fresh pancreatic juice at day 0.

Day	Trypsin	Chymotrypsin
0	100.0	100.0
4	99.2	103.7
68	92.1	84.6
70	92.1	95.8
75	92.4	91.9
82	89.1	90.0
104	83.0	82.5

Results summarized in table 2 show an almost similair decline of trypsin and chymotrypsin activities. At day 68 the chymotrypsin value is remarkable, it seems not to fit in the declining range.

3.2 The effect of freeze drying and storage of pancreas on trypsin

Before freezing and freeze drying, samples were taken at three different places of the pancreases. There were no significant differences between the activities in those three sub-samples. Therefore the average was taken for comparision with the activity after freeze drying. By determination of the (freeze)dry matter content (weigth pancreas/weigth freeze dried pancreas) the trypsin activity per gram freeze dried tissue was corrected to the activity per gram (wet) pancreas. Between the analysis in fresh tissue and freeze dried tissue there was a time period of 14 days.

Table 3. Trypsin activity in pancreas before and after freeze drying, expressed in units per gram pancreas

Porcine id.no.	Trypsin activity before (U/g)	after (U/g)	% (*)
24	600	651	108.5
23	2516	2221	88.3
12	1911	2072	108.4

(*) =Trypsin activity in freeze dried pancreatic juice as a percentage of the activity in fresh pancreatic juice.

In this experiment no chymotrypsin analyses have been done. The differences between activities in fresh and freeze dried pancreas, might be related to the interassay variation of the analytical method. No investigation for interassay variation or comparability has been performed. One pancreas was freeze dried and thereafter stored at +4°C. After 245 days it was analysed, the results show a decrease in trypsin activity of 19.6%. The chymotrypsin activity decreased with 66%.

3.3 The effect of freeze drying and storage of ileum chyme on trypsin and chymotrypsin

In the original ileum chyme the activity of trypsine and chymotrypsine was determined (n = 5). Trypsin activity = 12102 U/kg (s.d.=239) and chymotrypsin activity = 2984 U/kg (s.d.=71).
At 14, 20 and 245 days after collection or freeze drying the activity of both enzymes were determined and corrected to the activity in the original chyme. The results were compared with the results found at day 0 and expressed as a percentage of those actvities.

Table 4. Trypsin en chymotrypsin activity in ileum chyme after freeze drying and storage at ambient temperature (under dry conditions). Activities as a percentage of activity in fresh ileum chyme at day 0.

Day	Trypsin	Chymotrypsin
0	100.0	100.0
14	111.0	140.8
20	105.7	129.2
245	131.5	145.8

No decrease of activites, even after 245 days storage, has been found. The increase of chymotrypsin activity cannot be explained. An invalid analysis at day 0 might be the cause to this apparent increase.
The difference between the trypsin activity at day 0 and day 14 or 20 is probably due to the variability of the medhod. After 245 days the trypsin activity was found to be 31.5 % higher as at day 0. Except variations caused by the analytical method, the unexpected presence of zymogens, activated by trypsine, may be the cause of this increase.

4. Conclusions

An inadequate storage procedure will lead to invalid results and might led to scientifical misinterpretation. When biological samples are not treated and stored under appropiate conditions, considerable changes of proteolitic enzyme activities will appear.
Working with frozen pancreatic juice and tissue can give unpredictable changes in activity just during thawing. A possible solution to this problem might be the use of freeze dried material. A freeze dried sample-pool can be used as a control/reference sample to check compariability of the results from different laboratories, or to check day-to-day variances within one laboratory. The experiments described, indicate that freeze drying may provide considerable changes of enzym activities during several months. The experiments were ment as "pilot experiments" just to get basic information about the possiblities of freeze drying in relation to enzym activitys. For definite results and conclusions about the effect of freeze drying and storage more experiments with more samples will be needed. Experiments are planned in near future.

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COMPARISON OF THE RATE OF PASSAGE OF DIGESTA IN PIGS MODIFIED BY ILEO-RECTAL ANASTOMOSIS OR FITTED WITH AN ILEAL T-CANNULA

P. Leterme, L. Pirard, A. Théwis and E. François[1]

Faculté des Sciences agronomiques. UER de Zootechnie B-5030 GEMBLOUX (Belgium)
[1] Ministère de l'Agriculture. Station de Chimie et Physique agricoles B-5030 GEMBLOUX (Belgium)

Abstract

The rate of passage of digesta through the digestive tract of pigs with an ileo-rectal anastomosis (IRA) or an ileal T-cannula was measured according to the single marker dose method by using polyethylene glycol (PEG) as liquid marker, Cr_2O_3 as solid phase marker and Yb_2O_3-mordanted cell walls as fibre marker. The rate of passage was estimated by the slope of the regression equations of ln of marker concentration with time. PEG was eliminated a lot faster from the digestive tract of both kinds of pigs than the solid phase markers, while Cr_2O_3 was retained a little longer than Yb_2O_3. Both Cr_2O_3 and Yb_2O_3 passed through the gut of IRA pigs faster than through that of cannulated pigs but the difference was only significant ($P < 0.05$) for Cr_2O_3. The results are discussed and hypotheses are put forward to explain the differences observed between both kinds of pigs.

Introduction

Ileal analysis is the most commonly used method for the determination of amino acid digestibilities in feedstuffs for pigs but it requires the use of fistulated pigs. The problems linked to the use of cannulas (simple or re-entrant) to collect the digesta are well known : difficulty to obtain representative samples of digesta, blockages in the cannulas, etc. Ileo-rectal anastomosis (IRA) has been proposed as an alternative method to cannulation in order to overcome these problems (Laplace et al., 1985). That technique needs to be validated by comparison with a fistulation technique. For that purpose, two experiments were recently carried out : the first one to compare IRA to the use of T-cannula (Leterme et al., 1990), the other one to compare IRA to the ileo-colic post-valve (ICPV) fistulation (Darcy and Laplace, 1990). In both cases, the digestibilities observed were lower with the IRA technique. With the surgical IRA procedure, the ileo-caeco-colic sphincter is not preserved. We could thus wonder whether the absence of the sphincter modified the transit time of the digesta in the pig's small intestine, thereby decreasing the digestibility of the nutrients.

Accordingly, the aim of the present work was to compare, by use of markers, the transit time of digesta in the gastro-intestinal tract of IRA pigs or pigs fitted with an ileal T-cannula. As the practical needs of passage studies make it desirable to have measurements of both liquid and solid fractions of the digesta, we used polyethylene glycol (PEG) as a liquid marker and chromic oxide (Cr_2O_3) as a solid marker. In an attempt to study the behaviour of the fibrous fraction, we labelled barley cell walls by the mordanting procedure with a rare earth oxide : ytterbium oxide (Yb_2O_3). Those mordanted fibres were also included in the proof diet of the pigs.

Materials and Methods

Eight Belgian Landrace Hn male pigs (initial weight : 42 ± 2 kg) were used. Four were fitted with a T-cannula (internal Ø : 18 mm; 10 cm anterior to the ileo-colic valve). IRA was performed on the 4 other pigs according to the surgical method described by Green et al. (1987). Ten days before the experiment, the pigs were fed twice daily (8-16 h) with a basal diet (66 % barley, 30 % peas and 4 % min./vit. premix) at the rate of 60 g DM/kg

$W^{0.75}$. In order to compensate for the bypass of the large intestine, IRA pigs were continuously allowed extra water, NaCl (7.5 $g.day^{-1}$), $NaHCO_3$ (7.5 $g.day^{-1}$) and Na bisulfite menadione or vitamine K_3 (0.2 $g.day^{-1}$).

The test meal included 24 g PEG and 3 g Cr_2O_3/kg DM. Eight % of the diet (on a DM basis) were substituted by the Yb_2O_3-mordanted cell walls. The cell wall extraction of the barley seeds was realized by acetic hydrolysis (Pond et al., 1986). The cell walls were then soaked at 100 °C for 24 h in an hydrochloric solution of Yb_2O_3 (20 mg/g cell walls). The excess of Yb_2O_3 was recovered with a citric solution. The chemical composition of the diet was then as follows : 94 % of organic matter, 14.4 % of crude protein, 7.8 % of crude fibre, 23.8 % of NDF and 8.9 % of ADF.

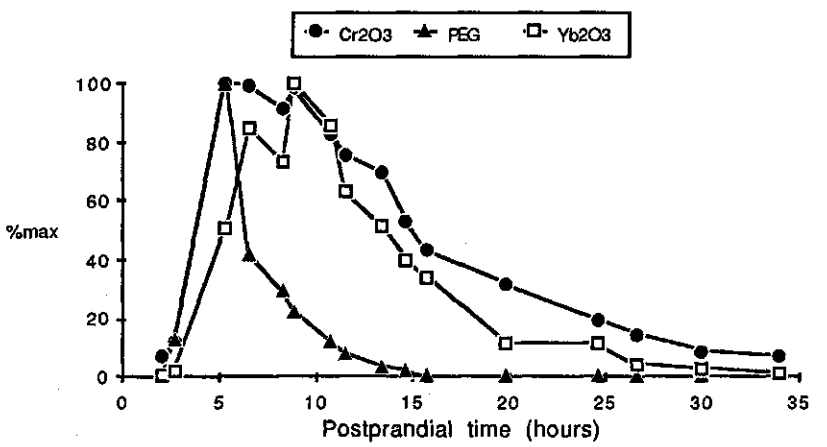
The unique experimental diet was given to the pigs the first day of the experiment at 8 a.m. The following diets were all constituted by the basal diet. For the IRA pigs, each discharge of digesta was collected and weighed and the hour was noted for 18 h post-prandial. Thereafter, the digesta were collected at intervals of 2 or 4 h. The mean time of the period was considered to be the hour of collect. For the cannulated pigs, the digesta were collected in plastic bags fixed to the cannula. When the plastic bag was filled with digesta in one emission, digesta were immediately collected, weighed and the hour of collect noted. When the discharge of digesta was progressive, the mean time of the period (max. 2 h for the first 24 h and max. 4 h thereafter) was noted as the hour of collect and the digesta were collected and weighed.

The digesta were then freeze-dried and ground through a 1 mm-mesh screen. Ytterbium was estimated by atomic absorption according to a modification of the method of Siddons et al. (1985), PEG by turbidimetry (Hyden, 1955) and chromium by titration with Mohr salt after a nitro-perchloric oxidization of the sample (François et al., 1978).

Results and Discussion

As an example, the excretion curves of the 3 markers at the ileal level of a cannulated and an IRA pig is given in figure 1. The postprandial increase of marker concentrations in the digesta is very fast. The decrease of PEG concentrations is also rapid, which means that the liquid fraction passed through the gut faster than the solid one.

a. IRA pig



b. Cannulated pig

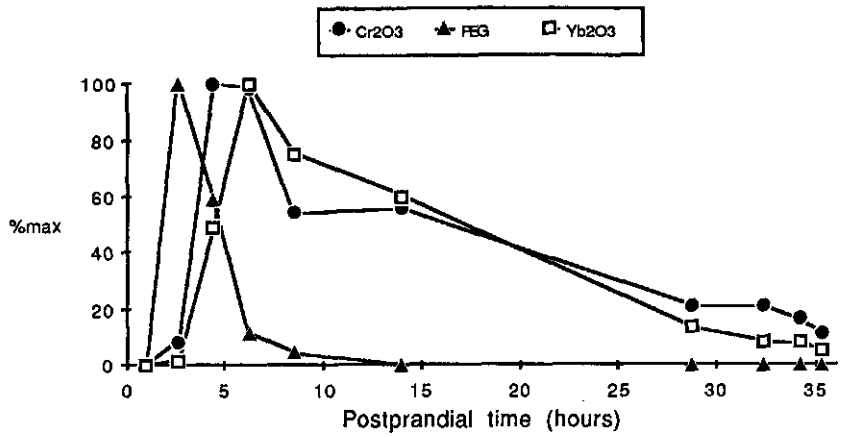


Figure 1. Evolution of the PEG, Cr_2O_3 and Yb_2O_3 concentration (% max.) in ileal digesta of cannulated and IRA pigs following feeding of a marked meal.

As Graham and Aman (1986) showed that fibres left the stomach 2 h 30 to 4 h 30 later than Cr_2O_3, we could wait for a slower evolution of the Yb_2O_3 concentration in comparison with Cr_2O_3. It was not the case here but we must remember that the mordanted cell walls were added to the diet and were thus not bound to the other feed components. On the other hand, the effect of fibres on intestine motility is well known.

The calculation of the mean retention time of the digesta in the digestive tract necessitates the adjustment of a mathematical function to the excretion profile of the markers. Some problems during the collect with the cannula limited the amount of samples and prevented us from calculating these functions. For the same reasons, the determination of the mean retention time by means of the method of moments was not possible (Thielemans et al., 1978). In order to compare the rate of passage of the markers between both kinds of pigs, we determined the regression equation of ln of marker concentration (expressed as a % of the maximum of excretion (Cm)) with time (t) : $\ln Cm = a + b.\ t$. The slope of the regression line (b) is an estimate of rate of passage. A fast decrease in marker concentration thus corresponds to a fast rate of passage.

The main parameters of the regression equations obtained for both kinds of pigs are presented in Table 1. The regression lines were obtained by assembling the data of all the pigs. The statistical analysis was realized with the regression line obtained for each pig and consisted in a test of parallelism of the regression lines with the *t* distribution of Student (Dagnelie, 1980). The data of one cannulated pig were discarded.

Table 1. Slope and dispersion parameters of the regression lines of ln of the marker concentration (% of max. concentration) with time.

	b			$\sigma_{Y.x}$			r		
	PEG	Cr_2O_3	Yb_2O_3	PEG	Cr_2O_3	Yb_2O_3	PEG	Cr_2O_3	Yb_2O_3
IRA pigs	- 0.36	- 0.09a	- 0.15	0.44	0.33	0.23	- 0.92	- 0.93	- 0.92
Cannulated pigs	- 0.38	- 0.07b	- 0.13	0.66	0.23	0.54	- 0.94	- 0.96	- 0.93

a, b : $P < 0.05$

The rate of passage of the fibrous fraction (Yb_2O_3) was faster than the rest of the solid fraction (Cr_2O_3), for the reasons given above. The rate of passage of Cr_2O_3 and Yb_2O_3 was faster for the IRA pigs but the difference is significative ($P < 0.05$) only for Cr_2O_3. In all cases, the variability ($\sigma_{Y.x}$) was very important. The faster transit time of digesta in the small intestine of the IRA pigs can explain the lower ileal digestibility observed previously in comparison with the cannulated pigs (Leterme et al., 1990). Nevertheless, Pond et al. (1986) found no influence of the digesta rate of passage on the nutrient digestibility but it only concerned the faecal digestibility of those nutrients.

Darcy-Vrillon and Laplace (1990) recently incriminated the lack of the ileo-colic valve function in IRA pigs because the valve influences the retention time of digesta in the small intestine. Ten years ago, these same authors showed that the digesta rate of passage in a pig small intestine was 60 to 90 minutes faster in pigs fitted with an ileal re-entrant cannula compared with an ICPV cannula (Darcy et al., 1980). But in cannulated pigs, we do not know if the sphincter can regulate the digesta rate of passage when the cannula, which is before the sphincter, is open. Moreover, Green (1988) showed that a 'post-valve' IRA did not improve the digestibility of N or amino acids.

We can also wonder whether there is not a 'feed-back' effect of the large intestine on the upper digestive tract which would modify the digestive secretions and in this way the digestibility of the nutrients. That hypothesis should nevertheless be confirmed.

The problems encountered during the collect of digesta in cannulated pigs (few digesta, long time between 2 collections, etc) may also have influenced the slopes of the regression lines. Besides, Laplace et al. (1983) demonstrated that the two main sources of variation in the rate of passage in a pig small intestine was the individual variation among pigs and the particular reaction of each pig to the proof diet. Further investigations should be undertaken with more pigs in order to compare both kinds of pigs in the best way.

As a conclusion, the digesta rate of passage in the small intestine is a little faster in IRA than in cannulated pigs, which can explain the small difference of digestibility previously observed between both kinds of pigs (Leterme et al., 1990). Nevertheless, new experiments should be performed to confirm the present data.

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SESSION 5
Digestion of carbohydrates in the pig

DIGESTION OF CARBOHYDRATES IN THE PIG

W.Drochner

Institute for Animal Nutrition,Veterinary School,Hanover,Federal Republic of Germany

Abstract

A review on carbohydrate digestion in the pig is given.The cascade of digestion in the mouth,stomach,small and large intestine is described.Principles of enzymatic and fermentative digestion according to new results with fistulated animals are discussed.The efficacy and quality of fermentation in the large intestine depending on level and quality of carbohydrates in the diet are demonstrated.Some aspects of energetical efficacy of hindgut digestion are discussed.

Keywords: carbohydrate digestion,soluble carbohydrates, starch,fiber,enzymes,fermentation,efficacy

Introduction

The carbohydrate-fraction in feeds is heterogenous:
-monosaccharides(glucose,fructose,mannose,pentoses)
-disaccharides (lactose,sucrose)
-oligosaccharides(raffinose et al.)
-polysaccharides(starch,inulin,cellulose,hemicellulose,pentosanes and pectic substances)
Thus a distinction between carbohydrates digested by enzymatic degradation and those which are fermented is helpfull. Longland et al.(1988) suggested the terminus non-starch polysaccharide including cellulose,hemicellulose,pectins but not lignin.The relation between enzymatic and fermentative digestion in different parts of the intestinal tract variates.Thus it will be rich in meaning to discuss carbohydrate digestion in correlation to its location in the intestine.

I.FEED INTAKE AND DIGESTION IN THE STOMACH

A)Feed intake

Increasing levels of crude fiber >6-7% of the diet reduce voluntary feed intake.Lower levels stimulate compensatory energy ingestion(Campbell & Taverner,1986,Drochner& Coenen 1986). Suckling pigs prefere diets with low fiber content.
Digestion in the mouth of the pig is neglectable.Intake of dry feed induces secretion of saliva ,containing water,mucus and alpha-amylase.Kudryavtsev(1935) and Kvasnitskii(1951)have shown,that the saliva of man,dog and pig is quite similar in composition.The enzyme-content depends on level of secretion, water-content of the diet age and corresponds roughly with the level of feed intake(Arkhipovets,1956).The breakdown-products of pig-alpha-amylase from saliva are maltose,maltotriose and some dextrins.The activity of saliva-amylase is de-

pendend on the presence of chlorine(Myrbäck,1926) for full activity within a pH-range of 3,8-9,4 with an optimum,in presence of chlorine ,of 6.9(Bernfeld et al.,1948),but the described conditions have been evaluated with human saliva.

B.Digestion in the stomach

1)Sugars and starches

A limited breakdown of sugars and starches in the stomach has been demonstrated,it is due to fermentation.This degradation results in formation of volatile fatty acids and lactic acid. The gastric mucosa is able to absorb limited amounts of scfa - but its efficiency is not comparable to that of the small intestine,cecum or colon(Argenzio and Southworth,1974).The capacity of the stomach flora to produce scfa has been proved to be as high as 150 mmol/l in slaughter pigs and 30 to 90 mmol/l in young piglets (Friend et al.,1963).In the first weeks of life,lactic acid is the most prominent scfa,due to the lactose content of sow's milk.Its distri bution is not homogenous:Slivitskii(1973) found the highest concentrations in the dorsal part of gastric contents with pH-levels near to 3.There is a postprandial rhythm:the concentration falls for a short period (Cranwell et al.,1968) and then increases for a time of ten to 12 hours(depending on feeding interval1) (Etienne,1971).The intensity of the gastric fermentation is due to adaptation,availabilty of carbohydrates and pH-level. Especially an inverse correllation between HCl-secretion and lactic acid production has been demonstrated by Cranwell et al.(1976)in the suckling piglet.The influence of age on lactic acid fermentation has been shown by Friend et al.(1963). They demonstrated high levels of this acid in the very young suckling piglet and increasing amounts of scfa in older pigs parallel to a reduction of the pH of the gastric contents. Substrates of fermentation in suckling piglets or young early weaned pigs are monosaccharides,lactose,sucrose and even degradation products of starch,formed by saliva-amylase.A limited formation of organic acids from hemicellulose and pectic substances as well as from alpha-limit-dextrins (branched chains from terminal starch units after amylase-splitting) is neglectable.High levels of enzymatically undigestible carbohydrates(as for instance cellulose) rarely are found in the diets of suckling piglets.They show however interactions with the gastral fermentative process in weaning pigs (retardation of the formation of scfa,buffering effects (Drochner & Coenen,1986)).Newborn piglets show a quite high pH in the stomach,thus,the "colonisation" of the gastrointestinal tract with bacteria is possible.Subsequently,the barrier-function of the stomach is maintained by low pH-levels, formed by lactic acid fermentation and step by step increasing amounts of secreted HCl.The type of diet fed in the period of weaning is responsible for the amount of lactic acid formation and level of HCl-secretion.A postprandial pH-rhythm was demonstrated by Seve and Laplace(1975),an age dependency by Schnabel et al.(1982).Cereal diets in exchange to lactose-containing diets induce a continuous reduction of the formation of lactic acid and an augmentation of the concen-

tration of volatile fatty acids (Friend et al.,1963).

2).Enzymatically undigestible carbohydrates

Digestion of carbohydrates of fiber- or cell-wall-typus in the stomach is limited . Small amounts of hemicelluloses might be fermented as well as small amounts of pectic substances(Drochner,1984).A certain break-down of hemicelluloses in the stomach has been demonstrated by Keys and DeBarthe,1974.This break down seems to be of bacterial origin,but a direct hydrolytic split off of some carbohydrate-molecules from the polymer chain under low-pH-conditions is discussed as well.The cited authors as well demonstrated,that the type of fiber and perhaps the lignin-coating is responsible for the intensity of digestion.Additionally, a selected separation of soluble ingredients of the diet proceeds causing a retention of fiber-rich material in the stomach. This reservoir partly remains in the stomach till the next feeding time.So pH-levels in this cavity remain low as feed -after beeing swallowed- is mixed with a low-pH-chyme.See tab.Nr.1.Saliva-volume increases,depending on fiber content of the diet(Zebrowska and Low,1989)(tab.2).

Tab.1:Postprandial pH-values(fundus) feeding diets rich in fiber(Z=6% crude fiber) or controll(A)

	A	Z
time		
7.45	2,19(0,17)	2,50(0,40)
feeding 8.00		
8.15	3,94(1,76)	2,52(0,12)
9.00	3,75(1,95)	2,88(0,91)
10.00	3,80(1,22)	2,49(0,25)
11.00	3,49(0,98)	2,29(0,17)*
12.00	3,23(1,02)	2,91(0,83)
13.00	2,87(0,70)	2,58(0,60)
14.00	2,32(0,29)	2,44(0,47)
15.00	2,20(0,31)	2,42(0,21)

n=5,coefficient of variation in brackets
(Drochner & Coenen,1986)

Tab.2:Secretion of saliva/gastric mucus,bile and pancreatic chyme in pigs (40 kg live weight,24 hours)feeding a high-fiber diet(B) compared to a control

	diet A(5% NDR)	diet B(18% NDR)
saliva/gastric mucus	4,0 l	8,0 l
bile	1,2 l	1,7 l
pancreatic chyme	1,2 l	2,2 l

(data from Zebrowska and Low,1987)

II.DIGESTION IN THE SMALL INTESTINE

A.Non-fiber-carbohydrates

The digesta-carbohydrates entering the small intestine are digested in different ways.Monosaccharides as glucose are absorbed quite quickly whereas different monosaccharides-as for instance fructose- show a more or less retarded absorption.At the end of the ileum,the concentration of glucose in the digesta is extremely low(Drochner,1984) but small levels of fructose can be found.Thus glucose is -concerning to its absorbability- the ideal energy supply in animals with disturbed absorptive capacity.From very young piglets- according to Johnson(1949)- fructose and -according to Wise et al. (1954)- xylose are not well tolerated.
Disaccharides normally are split to monosaccharides preceeding the absorptive process and only traces can be absorbed directly by the mucosa(Täufel et al.,1967).Quality and quantity of the "disaccharide-digestive-process" is dependend on the activity of the corresponding enzymes.
The lactase acitivity in very young piglets is high.It is located in the brush border of the small intestine.According to Sato and Yamashina(1974),several enzymes with beta-galactosidase-activity can be found;the digestive enzyme hydrolyses different types of beta-galactosides,normally it is named brush-border-lactase.ThepH-optimum is near to 6,the concentration decreases with with age(Plimmer,1907).This knowledge has been confirmed in the last years with detailed studies and differentiated methods(including blockage of internal cellular lactases which are non-digestive enzymes).
In detailed studies Manners and Stevens(1972)showed,that the fall in activity happend predomimantly in the first week and subsequently was slow and mostly located to the end of the small intestine.In this connection a result of a Swedish group of scientists should be mentioned(Ekstrom et al.,1975). They found,that lactase levels of 21 day old piglets were merelyaffected by a lactose-containing diet but by genetical aspects and breed differences.
Since 1880(Brown & Heron),it is well known,that pig intestinal mucosa shows maltase- and sucrase-activities,which have been classified in recent years,namely by Dahlquist(1962). These maltases should be differentiated into:
-isomaltase,splitting isomaltose and some limit dextrins,resulting from alpha-amylase-splitting of starches,
-sucrase,splitting sucrose into fructose and glucose
-glucoamylase I,splitting dextrins,certain starches,isomaltose and limit dextrins,
-glucoamylase II ,a quite heat resistant maltase,with properties comparable to those of glucoamylase I .
Normally isomaltase and sucrase are correlated strictly,a so-called double molecule therefore has been discussed(Kidder and Manners,1976).The pH-optimum for this complex is limited to the small space from 6,0 to 6,5.The glucoamylases tolerate a broader spectrum from 6,5-7,5(Dahlquist,1960). The activity of sucrase is quite high except in newborn and very young piglets(Manners and Stevens,1972). Thus tolerance

of young piglets for sucrose is limited.In the first quarter of the small intestine,the maximum in artificially reared piglets can be measured between the second and third week of life,in lower parts of the intestinum(second quarter,this maximum rises up in 4 weeks old piglets.A certain rise of activity,esp.in the first and second quarter of the small intestine can be observed even in two or three years old pigs.The level in ileal mucosa remains quite low.
These findigs are a confirmation of older observations,that piglet performance on sucrose diets in the first days of life is very poor(Becker et al,1954).
Comparable to those results,total maltase activity rose in correlation to sucrase.Obviously not only age but even -to a small degree -diet,esp. starch and sugar content of the feed, might be responsible for high activities of the corresponding enzymes in the young piglet.High individual variations between members of one litter were found by Manners and Stevens(1972).Additionally the concentration of mucosal enzymes is dependend on gut-dilatation and feed content of sections of the intestinal tract(Stevens and Kidder,1972).In dilated sections they found a lower level of sucrase and glucoamylase.In later published studies,Kidder and Manners(1978) showed, that different glucoamylases showed this depression as well.
The secretion of the pancreas predominantly performs starch digestion in the small intestine.The concentrations of alpha-amylase in this substrat act in the same way as has been described for saliva-amylase.

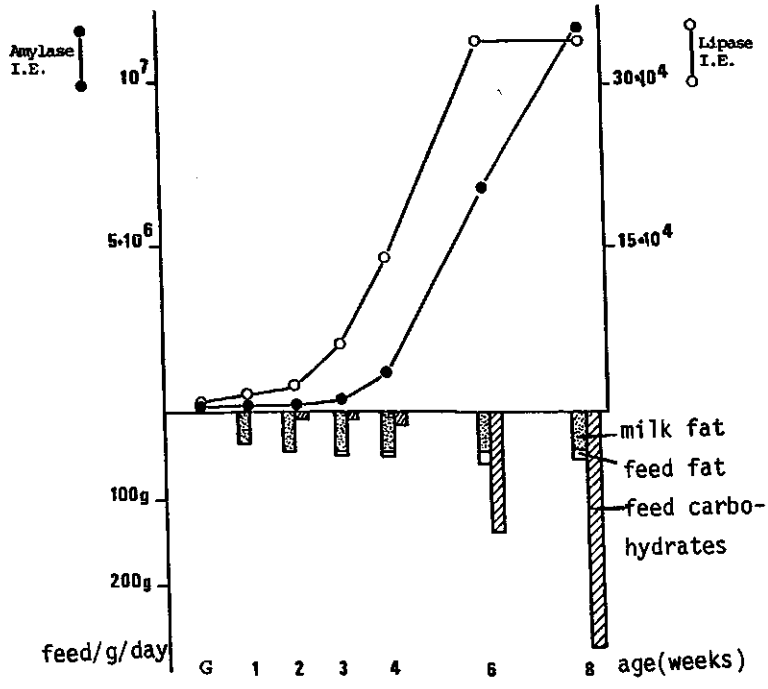

Fig.1 : Amylase and Lipase (activity) depending on age and type of feed(Aumaitre,1983)

The breakdown-products are:
maltose,alpha-limit-dextrin,maltotriose and some glucose.The dependency of pancreatic flow on fiber contents of the diet has been reviewed by Makking and Versteegen(1990) recently. The main variation factors are:age and starch content of the diet.As has been described by Corring et al.(1978),and Aumaitre(1983)-see fig.1- amylase-activity but not that of lipase can be induced by adaptation.It's surprising that alpha-amylase seems to be adsorbed to intestinal mucosa,a mechanism which cannot be interpretated now (Ruttloff et.al.,1967).The intensity of corn starch and lactose digestion in pigs variates.Rerat(1980) found large intraluminal amounts of maize and lactose 8 hours after feeding.He demonstrated a good concordance(fig.2) between postprandial gastric emptying after ingestion of starch(La-place,1979) and the corresponding arterio-porto-difference for reducing sugars demonstrating a certain"storage capacity" for highly available carbohydrates. That differences and blood flow measurements allowed a quantification of absorption of ingested reducing sugars(Rerat,A. A.,1985).He demonstrated,that the portal level of these sugars after ingestion of maize starch,glucose and sucrose,is comparably high in the first 3 hours after feed intake,but it is lower in older pigs,eating high levels of lactose(fig.2).

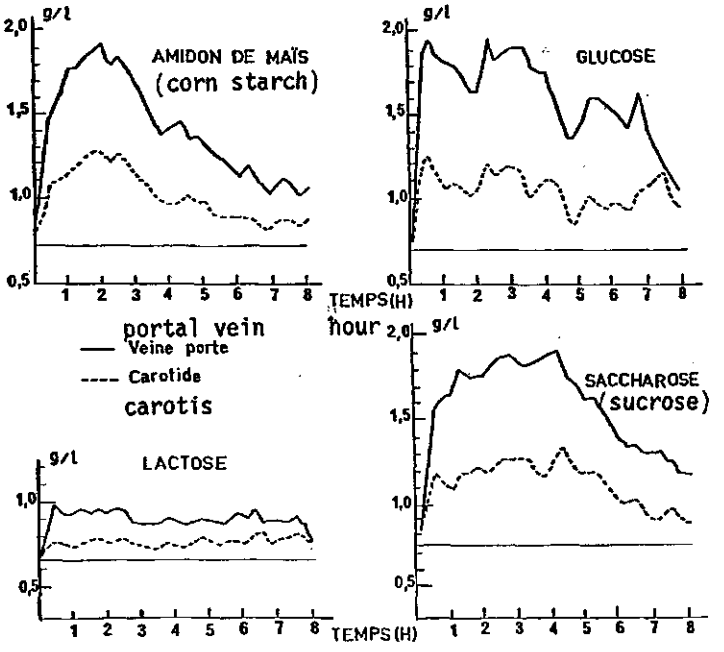

Fig.2:Postprandial cinetics of sugar concentrations in the blood after ingestion of different carbohydrates (n= 5) (Rerat,1981)

B.Carbohydrates of fiber-character

Prececal feed transit rises by addition of fiber or pectin to diets(Drochner,1984).It might be argued,that fibrous material binds water(colloidal,osmosis,van der Waal binding),affecting luminal viscosity.Interactions between mucosal membranes and digesta might affect hormonel feed back.Thus small intestine peristalsis might be retarded.Some aspects hitherto are discussed from Laplace and Roman(1979).A fermentative breakdown of carbohydrates during the passage of digesta through the small intestine might happen as well(tab.3).

Tab.3:Digestion of fiber in the small intestine of pigs

variant parameter	A	HZ	RZ	P
NDR	11,3(4,3)	2,0(0,8)***	5,8(4,8)**	20,2(2,7)***
hemicellulose	27,3(3,0)	12,2(3,5)***	12,0(4,8)***	42,5(3,5)***

A= low fiber-control,HZ= supplementation of 5% crude wood-product,RZ= 5% cellulose,P= 5% pectins;standard deviations in brackets;n=15;Drochner,1984

The total amount of prececal fermentation obviously is limited.On the other hand some scientists(Poppe et al.,1983 and Drochner,1984) found considerable amounts of microbial protein in ileal chyme,measured by determination of DaP(Di-amino-pimelic acid),which is a markeramino-acid for cell wall proteins of bacteria(fig.3).In total a prececal fermentation is evident.Millard and Chesson(1984) found a remarkable degradation of uronic acids(ca.50%) and of phenolic compounds from Brassica napus,Graham and Aman(1987) for peas and Longland et al.(1988) for sugar beet pulp.Longland et al(1989) found a remarkable break down of non-starch-polysaccharides in pigs with ileorectal shunts.

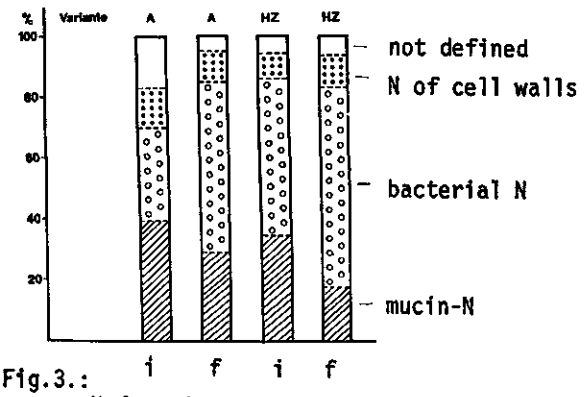

Fig.3.: N-fractions in ileal(i) and fecal digesta(f) feeding low and high (HZ) fiber diets(7% crude fiber,n=6)

Some aspects concerning prececal fermentation are not clear:
- the level of DaP in cell walls of bacteria from pig ileal chyme is variating(Ahrens,1984),
- break down of bacteria passing through the small intestine causes an enrichment of cell walls at the end of the ileum, so estimation of bacterial proteins is affected,
- the level of breakdown of β-glucosidic bounds by these bacteria is unknown.

In total the prececal digestibility of cellulose is low,it is higher but limited for hemicellulose and pectic substances (tab. 3).Thus ,the concentration of scfa in ileal chyme of pigs is low(0,01-0,05 mmol/l liquid Drochner& Meyer,1990).

C.Interactions

The fiber content of a diet however is basis for some interactions in the prececal digestive process.The following aspects should be discussed in detail:
-prececal digestibility declines (organic matter and protein)
-ileocecal flow of digesta increases,partly due to higher water flow,depending on reduced prececal digestibility(fig 4),
-ileocecal flow of buffering components increases,
-volume of the small intestine enlarges.

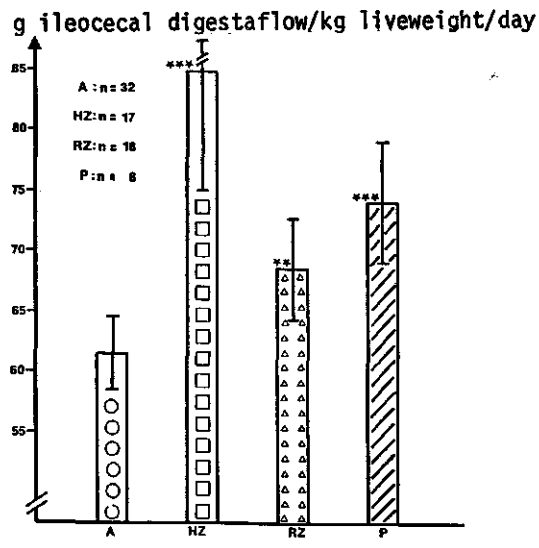

Fig.4.: Daily flow of digesta after variation of diet-fiber(A=control ,HZ= +5% crude wood product,R= +5% cellulose,P= + 5% pectins) (Drochner,1984)

The prececal apparent digestibility of the organic matter de-

clines correlated to increasing levels of fiber in the diet. The coefficient of regression is - 2,8(fig5)and thus by far higher than for the total digestive tract(-1,8).
The reasons for this decline of apparent prececal digestibility might be:
-coating of highly digestible material by fibrous material,
-stimulated endogenous secretions,
-reduced enzymatic break-down of nutrients(bulky fluid and direct binding of nutrients to fibers),
-differences in bacterial activities.

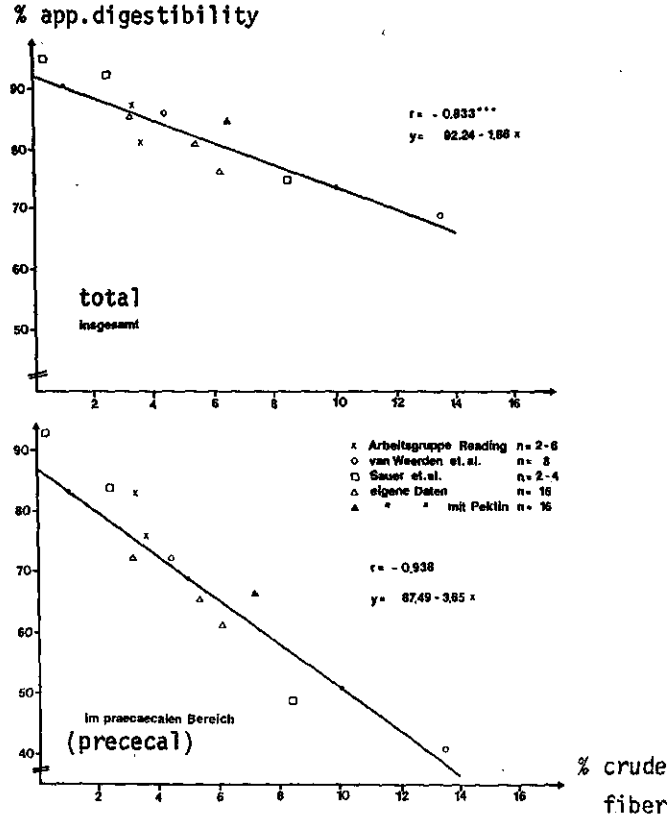

Fig.5: Prececal and postileal digestibility of organic matter with increasing fiber contents of the diet(Drochner,1984)

According to Maenhout et al.(1987),fiber-correlated reduction of prececal N-digestion is not due to stimulated trypsin-secretion.Low and Rainbird(1986) found however augmented levels of endogenous secretions after feeding guar gum.Zebrowska and Low(1987) documented a rise of pancreatic secretion with increasing levels of crude fiber.Indeed,the prececal N-digestibility declines(Drochner,1984) without any compensation in the colon.The reason for that is complex and not completely

understood. The following facts are discussed:
- cage effect(protein coating by fibrous material)
- increase of endogenous secretions and desquamations,
- rise of ileocecal urea flow,which is dependend from blood concentration and level of ileocecal flow of chyme,
- stimulated prececal bacterial protein synthesis (fig.3),
- direct binding of N and bile acids to fibers.

A lot of those listed points should be stated by additional experiments in the future.The correlation between intestinal N-loss and fiber-content of the diet however has been stated by Mangold and Behm in 1954.It has been evaluated by some other scientists,for instance Low(1989),who demonstrated,that increasing ileocecal flow of water,electrolytes,secretory products from liver,pancreas andmucosa cells correlates with increasing fiber levels in the diet.
The fiber -induced enlargement of the prececal intestinal tract has been described by Pekas(1986).He showed however, that an individual genetic disposition might be responsible for a broad variation .

III.DIGESTION OF CARBOHYDRATES IN THE LARGE INTESTINE

A.Non-fiber carbohydrates

Colon digestion is of fermentative character .Small amounts of enzymes seem to be degraded quickly after transit through the ileocecal valve.A lot of microorganisms have been shown to degrade cellulose and other fibers.A short review on this was given by Low et al.(1988).
Carbohydrates,flowing to the large intestine almost consist of polymers.Some starches(for instance from crude potatoes), small amounts of disaccharides(esp. lactose),and feed crude fiber pass the ileocecal valve and are fermented with different intensity.Small amounts of monosaccharides passing the ileocecal valve are fermented completely.The capacity of the large intestine to metabolize disaccharides as lactose as well is very high.In older pigs for lactose the main form of digestion seems to be fermentation(Rerat et al.,1983).In experiments(8 hour-periods)intracecally infused lactose(0,3 g/kg bm/h)was tolerated without deviations of fecal consistency .The tolerance for sucrose was even higher(Drochner et al. 1987b).Actually a flow as high as this level will not be present assuming normal feeding conditions.Feeding high levels of molasses (Ly et al.,1975) results in a remarkable flow of sucrose,and high amounts of whey(Kim et al.,1978)caused an intensive ileocecal flow of lactose to the large intestine , of slughter pigs,whereas young piglets show a high prececal lactose-digestion.Levels of starch,passing through the ileocecal valve are low with some exceptions:Crude potatoe starch and partly maize starch pass in higher amounts the ileocecal valve depending on the physical structure of the starches (Drochner,1988),whereas cooked potatoe starch is digested to high degrees- comparable to cereals in the small intestine. Mason and Just(1976) found quite low levels of digestion,but they used potatoe-starch which was heated at practical conditions.The amount of cereal starch,digested in the colon is normally less than 5% of the totaldigestion.The colonic di-

gestion of starch is performed by microbial alpha-amylase,derived from Cl.butyricum(etc.)(Whelan & Nasr,1951).Normally. the efficacy of postileal starch digestion is very high(>80% of crude potatoe starch,Rensing,1984).
An intensive microbial degradation occurs,resulting in a high production of :
-short chain fatty acids,
-carbon dioxide,
-hydrogen- and methane(Levitt et al.,1974),
-partly even of d- and l-lactic acid (Drochner et al.,1987a).
Exceeding the capacity of the colon an acidosis-like dysfermentation results:A decline of fecal pH and loss of the fecal consistency can be observed.This type of diarrhea has been classified by our research group as the"acidotic fermentative colonic diarrhea".
A comparable form of diarrhea might be observed after feeding diets with high levels of molasses,containing tri- and tetrasaccharides,being not absorbed in the small intestine(raffinose,stachyose),whey or of some crude starches (for instance crude potatoe starch).

B.Carbohydrates of fiber-character

Carbohydrates of fiber-type however will pass the ileócecal valve nearly entirely.As was discussed before,the pectin-hemicellulose-fraction in pigs prececally to some degree is fermented opposite to cellulose,which is not degraded.On the other hand the postileal digestibility of it however is by far higher than that of isolated or lignin-coated cellulose.
Cellulose is fermented by splitting the terminal sugar from the polymer step by step.Thus cellulose fermentation is time-consuming(van Soest,1982)and dependent on:
- transit time,
- adaptation(age)(availability of microbial enzymes),
- particle size,physical and chemical pretreatment,type of cellulose(for instance wood or straw)(conformation of the polymers highly branched=more soluble;degree of polymerization high=low solubility),
- coating of cellulose by lignin or silicates,
- individual factors,breed.

The high variation correlated to transit time is documented by experiments from Horszczaruk and Slijivovacki(1966),who used stripes of cellulose ,deponed into the cecum of fistulated pigs for different times.They showed,that cellulose-digestibility in the cecum is nearly complete,when time of incubation was sufficiently long.On the other hand it can be concluded,that increasing amounts of ingested crude fiber in a diet reduce apparent digestibilities of cellulose in the colon. Actually,Henry and Etienne(1969),Schneider and Kirchgessner(1977),Kass et al.(1980 and Gargallo and Zimmmerman(1980) found a distinct negative correlation between fiber intake level and apparent digestibility.
High levels of cellulose favour the development of a cellulolytic flora in the large intestine(van Soest,1982).Additionally ,the volume of the large intestine increases parallel to fiber-level of the diet(Drochner und Coenen,1987).This interaction was stated by Henkel(1977) as well.Passage time and

volume of the colon are dependend on oneanother,so a polyfactorial analysis is difficult and complex(this has been demonstrated by Auffray,Martinet and Rerat(1967)).The factor of age partly is identical with adpatation,volume of the colon and interfering transit time,an interpretation,which has been discussed already by Kellner(1916).In contrast to these factors,the age for it's one has only very small effects(Rerat, 1978);but Horszczaruk and Slijvovacki(1971)demonstrated,that cellulose break down in very young piglets was extremely low. Differences between cellulose from wood and straw show(Henkel,1977),that structure and type of cellulose are factors, influencing the digestibility of this material in the large intestine(Henkel & Schulz,1980).These differences were described already by Breirem et al(1958) and later on stated by Kupke and Henkel(1977).A recent study from Robertson et al. (1987)with the nylon bag technique in the large intestine of pigs verifies the well known differencens in digestibility between vegetable and cereal fibers.

Digestibility of lignin and silicates in the large intestine is near to zero,which means,that large-intestine bacteria are not able to degrade lignin in noticable amounts.A coating of cellulosic material by lignin will thus diminish bacterial access to this nutrient.

The particle size and physico-chemical treatments of cellulose can affect fiber digestibility in the large intestine.An incubation of cellulose with alcali and additional fine grinding resulted in high digestibilities(Woodman and Evans, 1947).A comparable result was obtained by Bergner and Betzin (1979),who developed a procedure to incubate straw-meal with hydrochloric acid.The acid treatment has the additional effect,that pH-levels in the stomach are reduced,the microbial quality of the fiber is excellent and the diarrhea disposition of weaning piglets declines,resulting in rising daily weight gain.The effects of treated straw meal on fecal and ileal digestibilties have been evaluated by Partridge et al.(1986).

In contrast to celluloses,the digestibility of isolated pectins in the large intestine of pigs is very high(80-90%).An as high degradation of pectic substances in the pig large intestine was shown by several authors(Albers and Henkel,1970, DeWilde,1980).This as well has been confirmed by Drochner (1984)by means of digestibility trials with reentrant-cannulated pigs.Additionally Kirchgessner et al.(1987) demonstrated in pigs fitted with cecal fistulas,that intracecally infused pectins were fermented in the large intestine to a very high degree.The effects of pectic substances in the large intestine in some respects are different from those of cellulose.According to the high degradability of this matter, the fecal volume onely moderately ist enlarged. The passagetime is nearly unchanged,the fecal dry matter is higher than after feeding cellulose.

Differences between breeds have been described by Fevrier et al.(1988),documenting a higher digestive capacity for fiber in Chinese Meishan pigs than in Large Whites.

C.Interactions

1.Microorganisms

The diet-effect on quality and quantity of microbial development in the colon recently has been studied by Varel(1987). He confirmed,that the intensity of large-intestine-fermentation is dependend on total number and type of microbial population.High fiber rations only moderately affect the total number of microorganisms but fiber degrading bacteria are favourized ,replacing others.The rise in fibrolytic organisms correlates with elevated cellulase-and xylanase activities in growing and adult pigs.Bacteroides succinogenes and ruminococcus flavefaciens predominantly seem to be favourized by related diets.Generally there is a striking resemblance of colon microflora to that of the rumen.So some sientists regard the colon as a special form of rumen.Pond(1987)looking to the future perspectives of scientific research in microbial digestion,discusses the possibility of introducing recombinant DNA in cloning techniques for cellulase genes perhaps for xylanase and even for ligninase..
On the other hand our basis knowledge on effiency of energy supply from large intestine digestion is not sufficient to point out the biological limitations.
Reviews on microbial biology of the large intestine of pigs have been published recently by several scientists(Allison, 1989,Fonty& Gouet,1989,Savage,1989,Ducluzeau & Raibaud,1989).

2.Final products of microbial fermentation

a)Concentration and level of production of vfa

The final products of carbohydrate-fermentation in the large intestine are energy containing: short chain fatty acids,lactic acid,methane or free of available energy:carbondioxide, hydrogen,water and heat.Concentration and level of scfa-production are dependend on degradability of the substrate,microbial adaptation and absorption(dependend on pH).But v.Engelhardt et al.(1989) found a low dependency on pH as the constant pH microclimate at the epithelial surface seems to be responsible for the efficiency of absorption.
The adaptation in high breed pigs seems to be excellent,in feral pigs however quite limited(Rose et al,1987).
Di- or oligosaccharides are highly attacked by colonic microflora,so that a quick and intensive production of scfa can be expected(Drochner et al,1987a).Pectic substances show a more retarded microbial break down(Drochner et al.1984).Cellulose is degraded step by step by"end to end"hydrolysis.This means, that the degradation proceeds slowly.Thus resulting concentrations of scfa are moderate(0,1-0,12 Mol/l liquid),especially when ileocecally flowing digesta are rich in cellulose(Friend et al.,1963).Indeed,Münchow and Häger(1989) found a reduction of the production of volatile fatty acids after feeding 10% rye straw meal untreated or treated with hydrochloric acid compared with a low fiber control.
The scfa-level in the intestine variates according to the location: in the stomach quite low ,in the small intestine 1/10th of that in the large intestine. Concentrations of scfa in the cecum variate with the rhythm of digestion depending

on feed intake time(Drochner,1984).The highest levels of scfa are present in the colon especially there,were the passage of digesta slows down markedly(Clemens et al,1974)(partly more than 200 umol/l of digesta).
The absorptive capacity of the large intestine of pigs for short chain fatty acids is very high. Experiments with cannulated pigs,receiving short chain fatty acids via the cecal cannula tolerated 2 mmol acetic acid per kg body weight,infused by one single dosage.Increasing dry matter contents of the feces were observed.The acetic acid was absorbed or metabolized completely(Drochner,1987a).
The volatile fatty acids seem to be utilized at the tissue level with a reduced efficacy.Acetic acid for instance,fed to rats,showed a by 15% reduced nutritive value for maintenance and growth compared to equivalent levels of starch(Veromel, 1968).This might be caused by a reduced uptake of portal acetic acid-compared to c3 and c4 by the liver(Rerat et al,1987). Additionally,this author found,that-in relation to the butyric acid content of colonic digesta-the corresponding concentration in the portal blood was much lower and that large amounts of energy were retained during their passage through the mucosa,confirming results of Imoto and Namioka(1978).High concentrations of circulating lactic acid are not correlated to intraluminal levels of this acid(Rerat et al.,1987).
The spectrum of vfa in cecal and colonal fluid depends on nutrients present in ileal chyme.As this substrate is rich in fiber(15-30% of dry matter),a predominating level of acetic acid is evident.Butyric- and propionic acid variate,according to ileocecal flow of starch,sugars and N (Drochner,et al., 1987a).Pectins seem to favour formation of C 4-acids.
Highly fermentable carbohydrates passing the ileocecal valve can cause dysfermentations with strict decline of pH and enrichment of d-/l-lactic acid(35 mmol/l and more)in the lumen (Drochner et al.,1987b).
The total level of scfa-production in the large intestine is still discussed.According to Marty and Demeyer(1973),theoretically 0,7 g scfa might result from fermentation of 1 g of organic matter.In vivo measurements of vfa-production by Kass et al.(1980)were performed by regression-calculation using increasing levels of dietary fiber.The results were a strict underestimation.Kennelly(1981)used isotope dilution techniques in the cecum tolerating the variation of cecal content and volume during postprandial rhythm.So the most direct access seems to be the measurement of portal blood flow with determinations of sugar and vfa concentrations(Rerat et al. 1985).Calculating a break-down of about 3 g organic matter per kg live weight and day in the pig large intestine,a level of 20 mmol/kg body mass and day would result.
Guisi and Rerat(1987) measured the portal flow of scfa,calculating an absorbtion of 15 mmol/kg liveweight and day after a ration with high amounts of alfalfa-meal(fig.6).

b)Energetical aspects of hindgut-digestion

Modelcalculations from Demeyer et al(1989) point out,that postileal fermentation of organic matter energetically might be more efficient than in the rumen(spectrum,loss of methane).

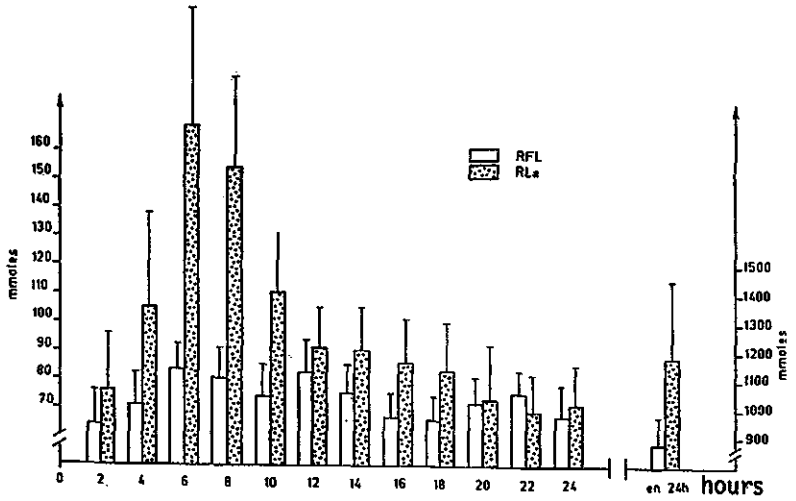

Fig 6 : Circadian variation of volatile fatty acid absorption after ingestion of a semi-synthetic diet containing 22% lucerne meal(RFL)(i.e.6% fibre)or 22% lactose and 6% purified cellulose(RLa).(Giusi et al.,1987)

Vervaeke et al.(1989) used three different approaches in order to estimate the value of postileal digestion: measuring production rates of volatile fatty acids in vitro; stoichiometric calculation from spectrum of scfa and amount of digested organic matter in vivo and in vitro;simulation of hindgut digestion using rumen flora.They found, that mean quantity of vfa produced in the hindgut was 10g/100 g feed intake.Percentage of nettoenergy resulting from hindgut fermentation in relation to total was calculated with 11,3%, Dierick et al.(1989).In 1990 this group confirmed(Dierick et al.,1990),that vfa contribute only to a small but remarkable extent to the maintenance requirements and partly for growth. They claim,that respiration and balance trials would be a prerequisite for netto-energy-estimations ,supported by carcass analysis.Dierick et al(1990)found only 50% of the theoretical level of scfa-production which could be shown in in vivo studies with pigs,fistulated at the cecum.
In growing pigs,the hindgut fermentation contributes up to 15% of the total digestion of the organic matter(Dierick et al.,1990),whereas this level can reach 50% in sows(Müller & Kirchgessner,1983 a,b).Regarding weight gain,the total digestibility shows higher correlations than prececal digestion (Livingstone & Fowler,1987,Laplace et al.,1989).
Vfa contribute essentially to the maintenance requirements of the gut itself.The level of energy used from the intestinal wall and supplied by intraluminal production of scfa has been estimated to amount to 70% of the maintenance requirements of this organ (Henning &Hird,1972;McNeil et al.,1978).According

to Rerat et al.(1987),the contribution of vfa to maintenance requirements was nearly 30%(animals of 60 kg live weight), when high levels of nutrients with low prececal and high postileal digestibility were fed.These levels correspond to an amount of 2,244 mol of scfa,flowing to the portal vein. Concerning practical swine feeding,the contribution of the large intestine for total energy supply will variate between 5 and 15% of the digestible energy,according to model calculations concerning disappearance of organic matter,concentrations of vfa in the portal blood,blood flow measurements and arterio-venous-differences (Rerat,1978).All cited papers demonstrate a high variation of energetical value of hindgut-digestion. Factors of variation are:
- spectrum of vfa in relation to composition of the diet.
- level of methane-production ,heat loss,
- maintenance requirements of the fiber-related hypertrophic gut(Drochner & Coenen,1987,Pond et al.1989,Pekas(1986),
- fiber related level of endogenous secretions,proliferation of epithelial cells(Sakata,1988)stimulated by scfa,
- depressive effect of crude fiber for prececal apparent digestibility of organic matter.

Just et al.(1983) showed,that the efficay of energy utilization-in terms of netto energy-falls as the proportion of hindgut fermentation raises.So for each unit digested in the hindgut,a reduction in efficiency of 0,8% must be calculated. The fecal loss of volatile fatty acids is less than 1% of the digestible energy(Drochner,1984,Kirchgessner et al,1987).

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EFFECT OF SOURCE AND LEVEL OF DIETARY FIBRE ON MICROBIAL FERMENTATION IN THE LARGE INTESTINE OF PIGS

Knud Erik Bach Knudsen & Bent Borg Jensen

National Institute of Animal Science, Animal Physiology and Biochemistry, Foulum, P.O. Box 39, DK-8830 Tjele, Denmark

Abstract

Present investigation was undertaken to study the effect of source and level of dietary fibre (DF) on microbial fermentation in the large intestine of pigs. The data confirm that the carbohydrates, in particular non-starch polysaccharides, are the main energy substrates for large intestine microbial fermentation. More than 80% of the substrates fermented in the large intestine derive from carbohydrate sources. In addition of being the key factor for the overall microbial fermentation, the source of DF also affects the ratio between the short chain fatty acids (SCFA) produced. It was demonstrated that total production of SCFA increased from approximately 500 mmol/d on a wheat flour diet (low DF) to approximately 2000 mmol/d on a oat bran diet (high DF), while butyric acid production increased from 35 mmol/d to 260 mmol/d on the same two diets.

Introduction

Microbial fermentation occurs to a varying degree in the gastrointestinal (GI) tract of most mammals including the pig and has important implications for nutrient assimilation (e.g. Mason & Just, 1976; Bach Knudsen et al. 1990). The substrates for large intestine fermentation are a wide variety of dietary carbohydrates (non-starch polysaccharides (NSP), starch, sugars), dietary and endogenous proteins, glycoproteins, mucopolysaccharides etc (e.g. Mason, 1984; Bach Knudsen & Hansen, 1990). During the course of microbial fermentation these substrates are broken down to short chain fatty acids (SCFA; acetic-, propionic-, butyric acids) and various gasses (CO_2, CH_4, H_2). The SCFA produced are rapidly absorbed from the gut lumen (Argenzio & Stevens, 1984), stimulate water and sodium absorption (Argenzio & Whipp, 1979) and play an important role for the overall energy metabolism (Mason, 1980; Bach Knudsen & Hansen, 1990). The SCFA absorbed might have different effects in the body. Butyrate is belived to have important implications for the metabolism, structure and function of the epithelia cells lining the large intestine (Sakata & Yajima, 1984) where it is the prefered fuel over glucose (Roediger, 1980), while propionate may modify hepatic metabolism (Chen et al. 1984).

In recent studies (Jensen, 1989; Bach Knudsen et al. 1990), we have shown that the adenine nucleotides (ATP, adenosine 5'-triphosphate; ADP, adenosine 5'-diphosphate; AMP, adenosine 5'-monophosphate), the energy couplers between catabolic and anabolic processes in all living cells, are usefull indicators for microbial activity in the GI tract of pigs. The aim of the present investigation was therefore to study the effect of source and level of dietary fibre (DF) on microbial activity and microbial density in the large intestine of pigs. As DF sources were used various wheat- and oat products varying in content and composition of DF (Bach Knudsen, 1991).

Materials and Methods

The experimental diets were prepared from refined wheat flour (WF) (low DF, control), wheat flour + wheat bran (WFWB), wheat flour + oat bran (WFOB), oat flour (OF), rolled oats + oat bran (ROOB) and oat bran (OB). The six experimental diets were tested in two series of experiments. Expr. 1 include the diets: WF, WFWB, WFOB, and ROOB, while Expr. 2 comprise the diets: OF and OB. The low DF control provided on the day of slaughtering 62 g DF/d, diet WFWB 106 g DF/d, diet WFOB 109 g DF/d, diet OF 113 g DF/d, diet ROOB 194 g DF/d and diet OB 283 g DF/d. The experimental diets were fed to a total of twenty four ileum cannulated pigs; sixteen pigs in Expr. 1 and eight pigs in Expr. 2 with four pigs on each diets. Digestibility of polysaccharides and other major constituents in the large intestine were measured in a balance trial lasting four weeks (Bach Knudsen, 1991). After finishing the balance experiment, the pigs were fed the morning ration and killed 4 h post feeding. Immediately after slaughtering, the GI tract was removed and separated by ligatures into twelve sections. These comprise the cranial and caudal halves of the stomach (S_1, S_2), three equal segments of the small intestine (SI_1, SI_2, SI_3), the caecum (Ce) and six segments of the colon (C_1, C_2, C_3, C_4, C_5, C_6). The total content of each GI segments was carefully collected for determination of dry matter, pH, adenine nucleotides and SCFA. In Expr. 2 was digesta also collected from S_1, SI_1, SI_2, SI_3, Ce and C_4 for counting of total culturable microorganism. The concentration of adenine nucleotides in digesta content was estimated by the luciferin-luciferase method (McElroy, 1947; Bach Knudsen et al. 1990), while analyses of SCFA was performed as described by Bach Knudsen & Hansen (1990) and pH as described by Bach Knudsen et al. (1990). Quantitative bacteriological analyses of the predominant bacteria were performed using non-selective and selective media and the Hungate technique (Jensen, 1989).

Results and Discussion

Quantification of the amount of nutrients which are digested within the large intestine of pigs fed the wheat- and oat based diets point to the carbohydrates as the most important energy substrates for the large intestine microbial fermentation (Table 1). The bulk (77-86%) of the substrates fermented within this GI segment derives from carbohydrate sources of which NSP was by far the most important. Moreover, while the amount of low-molecular weight carbohydrates (LMW-CHO) and starch fermented were independent of the DF intake, the amount of NSP fermented varied in respons to the DF intake amounting to approximately 40 g/d when feeding the DF depleted wheat flour diet (DF intake 62 g/d) and to 184 g /d when feeding the oat bran diets (DF intake 283 g/d). This has important implications for the microbial activity in the large intestine which increased significantly in respons to more substrates entering the large intestine (Figure 1). Hence, the amount of substrates fermented and the microbial activity in the large intestine are largely regulated by the dietary composition in particular the DF content of diets.

In agreement with recent studies at this Institute with pigs (Jensen, 1989), the microbial activity and density at the various sites of the pig GI tract varied substantially, as shown for the two diets in Expr. 2 (Figure 2). Moreover, although the density of micro-organisms in caecum and colon is quit constant (approximately 10^{10}), the decrease in ATP concentration from caecum and proximal colon to distal colon suggests a decrease in metabolic activity once the energy becomes limiting during the movement aborally through the large intestine. Studies with pure rumen bacteria (Forsberg & Lam, 1977) and with mixed populations from pigs caecum (B.B. Jensen, unpublished) in batch cultures have shown that the ATP pool size is higher

during early exponential growth but decreases rapidly during the stationary phase. The substrates in caecum and proximal colon with all diets are the readily fermentable carbohydrates such as starch and sugar residues, mucus, soluble NSP, etc. which are known to favour propionic producing micro-organism (Leng, 1969; Wolin, 1975). The consequence is a rapid proliferation, a decrease in pH and an increase in propionic acid levels. As digesta moves aborally it is depleted of readily fermentable energy sources. The substrate available in distal colon comprise, therefore, the most difficult degradable cell wall materials of the ligno-cellulose type (Bach Knudsen & Hansen, 1990). These conditions are known to favour acetic acid producing micro-organisms (Leng, 1969).

Table 1. Intake of dietary fibre and calculated digested nutrients (g/d) in the large intestine of pigs fed wheat- and oat based diets

Expr....	1				2	
	WF	WFWB	WFOB	ROOB	OF	OB
DF intake (g/d)	62	106	109	194	113	283
Nutrients (g/d)						
Nitrogen	2.2	2.5	2.9	4.6	2.9	3.5
Fat	0.6	0.8	0.5	8.8	6.5	10.8
LMW-CHO	8.0	10.1	16.8	2.0	0.6	0.4
Starch	3.5	7.5	17.5	42.7	15.0	10.8
NSP	40.1	60.0	55.2	106.3	61.4	183.8
Total CHO	51.6	77.8	89.5	151.0	77.0	195.0

DF = dietary fibre; LMW-CHO = low-molecular weight carbohydrates; NSP = non-starch polysaccharides; CHO = carbohydrates; WF = wheat flour; WFWB = wheat flour + wheat bran; WFOB = wheat flour + oat bran; ROOB = rolled oats + oat bran; OF = oat flour; OB = oat bran.

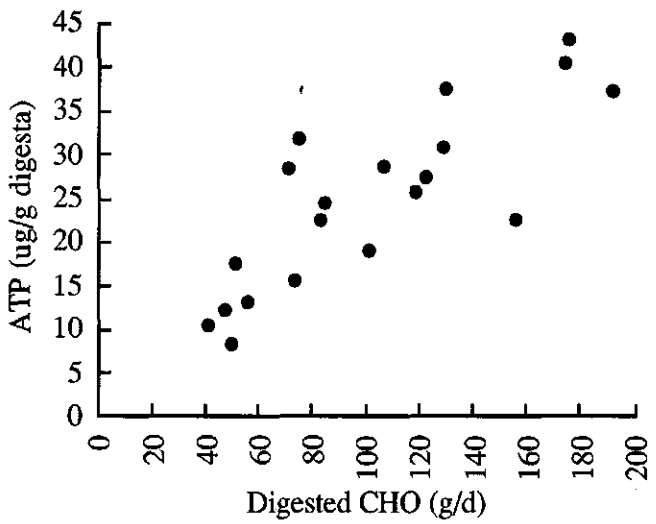

Figure 1. Correlation between the average ATP concentration in the large intestine and the amount of carbohydrate digested

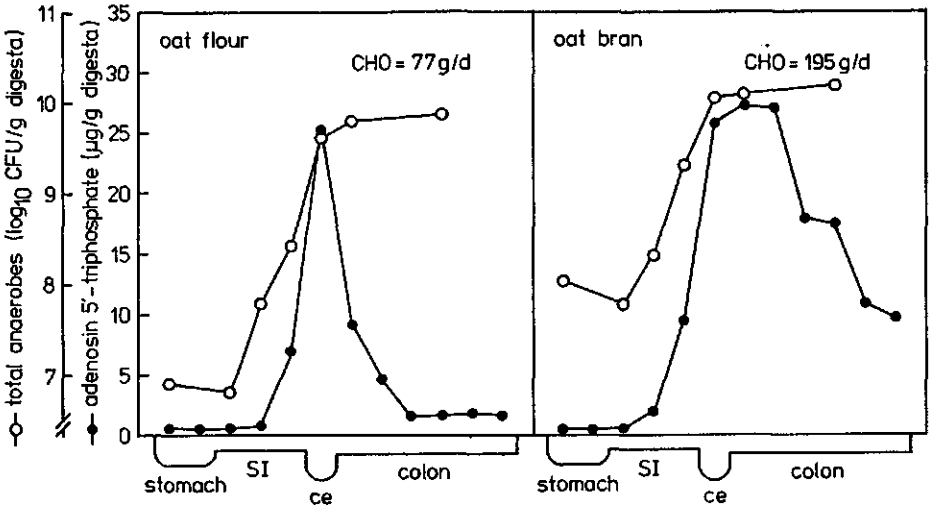

Figure 2. Adenosine 5'-triphosphate concentration and density of anaerobe microorganism of the gastrointestinal content of pigs fed the oat flour and oat bran diets in Expr. 2.

Table 2. Fermented carbohydrates, calculated production of short chain fatty acids and pH values in the large intestine of pigs fed wheat and oat based diets

Diet	WF	WFWB	OB	Ratio OB/WFWB
Fermented CHO (g/d)	52	78	195	2.5
SCFA produced (mmol/d)	520	780	1950	2.5
Acetic acid	322	423	936	2.2
Propionic acid	125	206	574	2.8
Butyric acid	31	62	260	4.2
pH	7.0	6.4	6.1	

CHO = carbohydrates; SCFA = short chain fatty acids; WF = wheat flour; WFWB = wheat flour + wheat bran; OB = oat bran.

The importance of fermentation for pigs and other simple-stomached animals, however, lies mainly in the type of products formed and their fate in the body. The major end products of fermentation, SCFA were the main anion in caecum and colon where they, independent of the dietary composition, were found in concentrations of 60-140 mmol/l in caecum and the two upper segments of colon (C_1-C_2) and from 35 mmol/l to 60 mmol/l in the lower portions of colon (C_3-C_6). Assuming that the equation for converting carbohydrate and proteins into SCFA given for man (Miller and Wolin, 1979; Macfarlane et al. 1986) are valid for pigs, the amount of fermented carbohydrates in theory leads to a production of approximately 500 mmol SCFA/d for diet WF to 2000 mmol SCFA/d for diet OB (Table 2) and the amount of fermented protein from approximately 36 mmol/d for diet WF to 75 mmol/d for diet ROOB. In addition there is an unknown quantity of lactic acid produced in the stomach and small intestine. Apart from providing the host with significant amounts of energy (Bach

Knudsen & Hansen, 1990) the SCFA produced might have more specific GI and metabolic effects (Cummings & Englyst, 1987). Butyrate, particularly, is considered to have important implications for metabolism, structure and function of epithelia cells lining the large intestine (Sakata and Yajima, 1984) where it is the preferred fuel over glucose (Roediger, 1980). Interestingly enough, DF addition and oat DF in particular not only causes an increase in butyric acid concentration in accordance with the overall SCFA, but type of DF source also affects the ratio of butyric acid relative to the other SCFA's. Hence, while the total production of SCFA increased from approximately 500 mmol/d for diet WF to 2000 mmol/d for diet OB; the increase in butyric acid production was from 35 mmol/d to 260 mmol/l for the two diets (Table 2). Certainly this is one of the most important factors to consider when discussing the mitogenic response in large intestine of certain DF sources (Lupton et al. 1988) and the heavier gut mucosa generally found in fibre-fed pigs (Kass et al. 1980).

The high proportion of ß-glucans in oat compared to wheat products (Bach Knudsen, 1991) encourage us to speculate whether this was responsible for the increased butyric acid production when feeding oat products. A thorough investigation of the composition of fermented NSP when feeding wheat flour and oat bran shows the following proportion of fermented polysaccharides in wheat flour: ß-glucans, 1%; cellulose, 6%; arabinoxylans, 78% and in oat bran: ß-glucans, 57%; cellulose, 7%; arabinoxylans 32%. However, we failed to verify this suggestion in a separate investigation. To a dietary fibre depleted wheat flour diet was added the same amount of DF in form of either oat bran, purified ß-glucans or the insoluble residues after extraction of ß-glucans. Mean butyric acid proportion of large intestine digesta was: wheat flour, 7%; wheat flour + ß-glucans, 7%; wheat flour + insoluble residues, 11% and wheat flour + oat bran, 10%. The results from this latter study, thus, indicate that the reason to the increased butyric acid production of oat products should be found in the insoluble residues consisting mainly of arabinoxylans and some insoluble ß-glucans.

Acknowledgements

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APPARENT ILEAL DIGESTIBILITY OF STARCH AND α GALACTOSIDES FROM PEAS BY EARLY WEANED PIGS: EFFECT OF EXTRUSION

J. Bengala Freire, A. Aumaitre, J. Peiniau and Y. Lebreton

Institut National de la Recherche Agronomique, Saint-Gilles, 35590 L'Hermitage, France

Abstract

Ileo-rectal anastomosis was tested to measure the apparent ileal digestibility (ID) of starch and α galactosides supplied by raw or extruded peas incorporated at the level of 45 % in the diet of weaned piglets. Termino-terminal procedure was finally choosen by comparison with the termino-lateral one, in order to prevent the microbial degradation caused by a backflow of digesta into the large intestine, demonstrated by the addition of a marker in the diet. ID of dietary nitrogen was affected by period or age of piglets and variety of peas ; it increased from 72.0 % to 78.6 % after extrusion of the seeds. ID of raw starch was lower in winter (94.4 %) than in spring peas (97.1 %) ; it increased from 95.8 % in raw to 99 % in extruded peas ; that of α galactosides averaged 75.7 % whatever the period, variety and treatment. Animals fed extruded peas had a higher postprandial serum glucose and insulin levels, and the amylase content and specific activity in their pancreas was increased by 70 % and 50 % , respectively.

Introduction

Peas are presently considered as a consistent alternative indigenous source of proteins for monogastric animals in Europe. They are characterized by the presence of 20 % of resistant starch (Colonna and Mercier, 1979), α galactosides (Cristofaro *et al.*, 1977 ; Besle *et al.*, 1981) and they contain various antinutritional factors (ANF_S), including antitryptic and antiamylase factors but also large amount of lectins (Huiseman, 1989; Bertrand *et al.*, (1988)). Dietary starch is digested in the small intestine of the pig (Bengala-Freire *et al.*, 1988), but partially hydrolysed and highly resistant granules could be further fermented in the large intestine by the microflora. In addition, α galactosides which are not hydrolyzed in the small intestine, are shown to cause deleterious fermentations in the hindgut as demonstrated in the rat by Cristofaro *et al.*, (1977).

Because of a low level of amylase in the pancreas (Corring *et al.*, 1978), the digestive potential of the early weaned piglet could be consequently altered by a diet containing fermentative pea carbohydrates (Graham *et al.*, 1989). Extrusion of peas, which destroys the physical structure of starch and ANF_S (Bertrand *et al.*, 1982), is assumed to be a valuable treatment to improve the digestive utilization of pea nutrients. Measurement of ileal digestibility in the pig proposed for amino acids (Darcy-Vrillon and Laplace, 1990), is of a great interest to characterize the digestive potential of the piglet towards polysaccharides. It has been particularly considered in the present experiment.

Moreover, the postprandial increase in glycaemia of the pig was reversely proportional to the molecular weight of dietary carbohydrate (Aumaître *et al.*, 1973). Large differences in the rate of intestinal breakdown of polysaccharides, and consequently monosaccharides absorption, were responsible for these results (Rérat *et al.*, 1984). Total amylase biosynthesis in the pancreas of the rat was also particularly stimulated by the ingestion of carbohydrates of low molecular weight such as glucose (Ben Abdeljlill and Desnuelle, 1964). These parameters should be of a further interest in case of early weaned piglets receiving large amounts of either raw starch or starch partially hydrolyzed after extrusion and mostly supplied by peas.

Materials and Methods

The methodological approach involved 3 groups of 6 piglets each weaned at 21 days of age. Total apparent digestibility (TD) was determined on group I (6 intact piglets) ; 6 animals of group II were fitted with a termino-lateral (TL) ileo-rectal anastomosis (Bengala-Freire *et al.*, 1988) and 6 animals of group III were fitted with a termino-terminal (TT) ileo-rectal anastomosis (Laplace *et al.*, 1985). Animals were pair-fed the same complex diet containing 0.5 % of titanous dioxide (TiO_2) as a marker; total collection of faeces was performed, TL and TT ileal digesta were collected continuously in cold

absolute alcohol to inhibit enzymes and microorganisms activities (Besle and Pitiot, 1976). Animals of group II were slaughtered and the content of their caecum, proximate and distal colon was sampled for dry matter and marker analysis (Figure 1).

The experiment was performed on 24 pair-fed male piglets from 6 litters and fitted with a TT ileo-rectal anastomosis. They were affected in six successive blocks to 4 treatments allowing a comparison between two varieties of peas incorporated at the level of 45 % in the diet and two treatments (raw and extruded for 30 seconds at 150°C). The starch content of feed and ileal effluents and the *in vitro* rate of hydrolysis of starch were measured on the two varieties of peas, before and after extrusion, using pancreatic juice of piglet according to the method proposed by Mercier (1968). Alpha-galactosides in feed were measured after extraction in uridine (0.4 mg/ml), deproteinization and centrifugation, by High Performance Liquid Chromatography. Extraction in ileal effluents required a further filtration on C 18 cartridge of Millipore SEP-PAK (Quemener, personnal communication). Serum glucose and insulin levels were measured on blood samples taken at the end of the experiment after an overnight fast, and 30 minutes after feeding (Aumaître *et al.*, 1973). Animals were slaughtered and the pancreas was removed for enzymatic essays (Corring *et al.*, 1978).

Table 1. Composition (1) and analysis of experimental diets (as DM)

Pea variety		Spring	Winter	Spring	Winter
Extrusion		-	-	+	+
Content :					
GE (MJ/kg)	(2)	18.9	18.9	19.08	19.05
CP (%)	(2)	25.7	25.5	26.0	25.8
DE (MJ/kg)	(3)	16.13	14.74	16.70	16.50
DP (%)	(3)	21.05	18.51	22.7	22.1
Starch (%)		49.3	50.2	51.3	48.6
α-D Galactosides % DM of diets					
Raffinose		0.59	0.94	0.22	0.30
Stachyose		1.71	1.08	1.98	0.96
Verbascose		1.26	2.11	1.58	2.99
ATF_S (IU/mg)	(4)	1.43	3.85	0	0

(1) Ingredients composition (%) of the diet was : Wheat : 36.4 ; Spring or Winter pea : 45.0 ; Soluble Fish Protein Concentrate (90 % CP) : 10.0 ; Vegetable oil : 4.0 ; L - Tryptophan : 0.10 ; TiO_2 : 0.50. Mineral, Trace elements and Vitamins : 4 (Bengala Freire et al., 1988).
(2) Gross Energy and Crude Protein (N x 6,25) respectively.
(3) Digestible Energy and Digestible Protein respectively.
(4) Antitryptic factors.

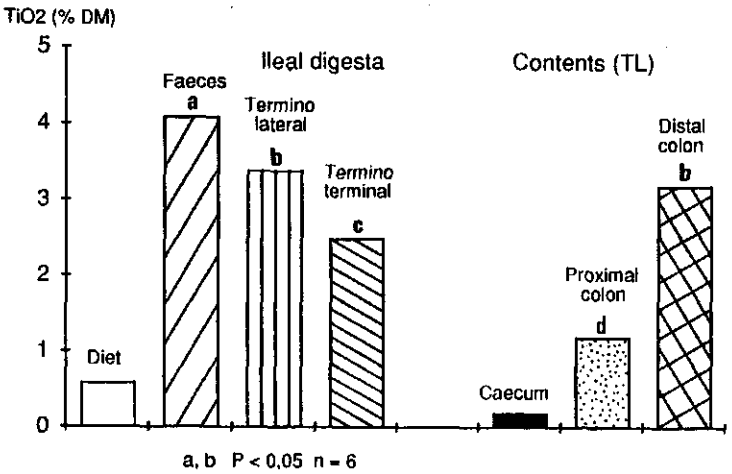
Results and Discussion

The content of the marker was higher in TL than in the TT excreta, both beeing significantly lower than in faeces. The level in the distal colon was high and not significantly different than that of the ileal effluent collected from termino-lateral procedure. It was significantly lower in the proximal colon, and very low in the caecum (Figure 1). These results indicated clearly that the TL anastomosis permitted a

backflow of the digesta in the large intestine as it was suspected by Bengala Freire *et al.*, (1988). Thereby the TL anastomosis was not suitable for measuring the ileal digestibility of fermentative carbohydrates (Graham *et al.*, 1989 ; Darcy Vrillon and Laplace, 1990).

Winter peas contained the same proportion of starch but higher amount of α galactosides, particularly verbascose, than the spring cultivar. The initial rate of hydrolysis of winter pea starch was similar to that of the wheat starch, but considerably higher (8–9 fold) for the spring pea starch. Extrusion doubled the initial rate of hydrolysis and increased by 50 % the percentage of starch hydrolyzed in 2 hours. Alpha–galactosides content was not affected by the treatment, but all the antitryptic factors were destroyed (Table 1). These results are in good agreement with those of Colonna and Mercier, (1979) for starch and those of Bertrand *et al.*, (1982) for antinutrional factors.

Figure 2. Percentage of marker (TiO2) at different levels of the digestive tract of the piglet : assessment of a technique for the expression of ileal digestibility



Compared with diets based on spring peas, TD of energy and of protein of the whole diet were reduced in the diet containing winter peas, leading to a lower content of Digestible Energy (DE) and Digestible Crude Protein (DP). Extrusion of both varieties of peas improved the TD of protein an dietary DP content (Table 1). ID of nitrogen was markedly improved (77.5 vs 71.2 % ; $P < 0.01$) between the first and the second week of the experiment i.e. between the 5 th and the 6 th week of age, in agreement with our previous observations (Aumaître, 1983 ; Bengala Freire *et al.*, 1988). It was lower for winter than for spring variety (72.0 vs 78.6 ; $P < 0.05$), despite the absence of the effect of pea lectin level on the brush border of the small intestine (Bertrand *et al.*, 1988). It was further increased by extrusion (76.6 vs 72.2 % ; $P < 0.05$) due to the destruction of ANF_S as suspected by Christison and Parra de Solano (1982).

ID of dietary starch was high, averaging 97.4 % in agreement with Graham et al, (1989) but affected by variety and extrusion, in agreement with Bertrand *et al.*, (1982) and Huisman, (1989). Assuming that the ileal digestibility of wheat starch, supplying 75 % of the total, was nearly 100 % in piglets (Bengala Freire *et al.*, 1988), one could conclude that starch of winter pea was particularly resistant to amylase in the small intestine, in good agreement with previous *in vitro* data. The value of ID of α galactosides was highly variable, and not affected by the treatments (Table 2). It was 2–fold higher than that found by Besle *et al.*, 1981 for the apparent digestibility of field beans α galactosides in the small intestine of the preruminant calf. It could be stressed on the presence of bacteria in the small intestine of the piglet, responsible for a partial breakdown, or on a backflow of bacteria from the rectum, induced by the surgical preparation (Darcy–Vrillon and Laplace, 1990).

Table 2. Apparent Ileal Digestibility (ID) of starch and α galactosides from peas ; effect of diet on pancreatic amylase and plasma insulin and glucose levels.

Variety Extrusion	Spring -	Winter -	Spring +	Winter +	Statistical analysis RSD	 Effects	
AID (%) of :							
Starch (1)	97.1	94.4	98.9	99.1	1.3	V**	E**
α Galactosides (2)	78.5	76.2	72.7	75.3	16.8	NS	NS
Nitrogen (3)	78.8	65.7	74.5	78.4	6.5	V*	E*
Insulin (μ IU/ml) (4)							
Fasting	<6	<6	<6	<6	-	-	-
30 mn	77^a	39^a	134^b	100^b	44	-	E*
Glucose mg/100 ml							
Fasting	95	99	104	105	12	-	-
30 mn	117^a	111^a	139^b	149^b	23	-	E**
Amylase (IU) (5)							
Total (x 10^3)	1448	2094	2988	3030	1031	-	E*
Specific	293	360	441	444	120	-	E*

(1) Significant effect of Variety V** $P < 0.01$, Extrusion E** $P < 0.01$ and period , and Interaction Variety x Extrusion, $P < 0.01$.
(2) α Galactosides = Raffinose + Stachyose + Verbascose
(3) Significant effect of period $P < 0.01$.
(4) Plasma insulin and glucose, measured before (F) and 30 minutes after test meal.
(5) Total (T) and specific activity as IU pancreas or IU/mg of protein/mn

The ingestion of easily degradable extruded starch was associated with higher levels of serum glucose and insulin and an increase in the activity of pancreatic amylase (Table 2). Postprandial hyperglycaemia already described by Aumaître *et al.*, (1973) and Rérat *et al.*, (1984), after an intake of starch derivative in pig was associated with a parallel increase in serum insulin level similar to that observed by Anderson, (1974) in atrial blood of the pig. Moreover in the rat, an increase in peripheric blood glucose stimulated the biosynthesis of pancreatic amylase (Ben Abdeljlill and Desnuelle, (1963). Favourable consequences on the ID of dietary starch and energy previously described could be generated by the stimulation of the biosynthesis of pancreatic amylase through insulin mediation, this suggesting a relationship between endocrine and exocrine secretions in the pancreas.
But, because of a dramatically low ID of nitrogen, mostly supplied by raw winter peas, one could suspect that favourable consequences of heat treatment (extrusion) were not only associated with the damage of starch, in good agreement with the hypothesis of Colonna, (1984). The destruction of lectin, particularly in the case of winter peas, could be beneficial despite any evidence of their attachement to the intestinal mucosa was provided (Bertrand *et al.*, 1988). Consequently, other ANFs, only characterized by trypsin inhibitors in the present study could be involved in the disturbance of the digestive or the metabolic processes.
In conclusion, the technique of terminno-terminal ileo-rectal anastomosis appeared to be a convenient method for the measurement of ID of dietary components in the weaned pig, and for the estimation of the digestive effect of ANFs of the peas. Postprandrial blood glucose and insulin levels together with amylase content in the pancreas could be indicators of beneficial effects of hydrothermal treatments on starch rich leguminous seeds.

Aknowledgement to the memory of the Late Robert Levrel for taking care of animals and collecting samples.

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DEFINITION AND ANALYSIS OF DIETARY FIBRE: EFFECT ON NUTRITIONAL EVALUATION

H. Graham[1,2], J. Inborr[1] & P. Aman[2]

[1]Finnfeeds International Ltd., Forum House, Redhill, Surrey RH1 6YS, England
[2]Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

Abstract

Dietary fibre is a group of chemically heterogeneous compounds differing widely in physical properties and physiological activity. In recent years methods for the analysis of dietary fibre, defined as the sum of non-starch polysaccharides (NSP) and Klason lignin, have been available. Use of these methods have illustrated that previous analytical techniques have underestimated both the content and degradability of fibre, leading to a misunderstanding of the dietary content and thus energy contribution of degradable fibre. However little is known of the physico-chemical properties of fibre polysaccharides or of the factors that control degradability.

Introduction

High fibre diets have long been associated with reduced digestibility in pigs, with Axelsson (1939) stating that a 1% increase in the crude fibre content of the diet will lead to a 1.7% decrease in organic matter digestibility in growing pigs. This can be attributed both to the presence of undegraded fibre and to the effect of fibre in reducing the apparent digestibility of other dietary components. The recent surge in interest in dietary fibre has resulted from the increased availability of high fibre feedstuffs, the development of accurate analytical methods and the realization that fibre can control the rate and extent of digestion and absorption other nutrients.

One major problem in the study of dietary fibre has been definition and analysis. However, dietary fibre is now defined either from a physiological point of view as the dietary components resistant to hydrolysis by mammalian enzymes or chemically as the sum of non-starch polysaccharides and Klason lignin (Theander & Aman, 1979). This latter definition has allowed the development of analytical methods for fibre.

Analytical Method

Briefly, the method for the analysis of dietary fibre as described by Theander & Aman (1979), later named the Uppsala method, is based on the prior removal of starch. A representative sample, usually 100-500 mg, of the ground material is incubated at pH 5 with a thermostable amylase (95°C, 1 h) and amyloglucosidase (60°C, overnight). Soluble fibres are then precipitated with addition of ethanol to 80% and total fibre recovered by centrifugation. Following drying at low temperature, the fibre residue is swollen in 12M sulfuric acid (30°C, 1 h) and hydrolyzed in 0.4M sulfuric acid (125°C, 1 h). Klason lignin, the acid insoluble residue, is removed by filtration and determined gravimetrically. The neutral NSP residues in the hydrolysate are reduced and acetylated before quantification by GLC. Uronic acid residues are estimated by decarboxylation in boiling hydriodic acid, with released carbon dioxide determined conductivimetrically. Alternatively uronic acid residues may be quantified spectrophotometrically (Englyst & Cummings, 1988).

As with any analytical technique, there are several critical points which determine the accuracy of the results. In the enzymatic step it is necessary to effect a complete degradation of the starch to oligomers soluble in 80% ethanol. Any residual starch will give an elevated content of glucose residues and thus of fibre, which is difficult to detect. The enzymes employed must not contain any fibre degrading activity under the assay conditions employed, and each enzyme batch should be tested for at least mixed-linked beta-glucanase and xylanase activities before use. The hydrolysis step is also rather critical, with under-hydrolysis giving high Klason lignin and low NSP values and over-treatment also giving low NSP values, particularly of xylose residues. Thus it is imperative that hydrolysis conditions, including the number of samples being processed, are kept constant. Incomplete reduction of the hydrolyzed monosaccharide residues is often the most common problem of the method, and this is manifested by a high apparent content of mannose residues and a low content of other sugars. This is often due to an incorrect reduction pH or an inactive reducing agent. It should also be kept in mind that the response of the GLC will change with column age, and thus correction factors to allow for losses during hydrolysis and derivization and for different GLC responses must be continually up-dated. It is obvious that this analytical procedure is rather sensitive, and it is advisable that even experienced analysts include at least one standard sample in all analytical runs to check starch degradation and fibre hydrolysis.

Table 1. The dietary fibre (DF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude fibre (CF) contents (g/kg DM) of some feedstuffs

	Content (g/kg DM)				As % of DF		
Feedstuff	DF	NDF	ADF	CF	NDF	ADF	CF
maize	94	82	22	20	91	23	21
wheat	108	98	32	23	91	30	21
barley	188	152	50	42	81	27	22
peas	163	104	71	52	64	44	32
soybean meal	241	151	88	76	66	37	32

Fibre in Pig Feeds

Fibre polysaccharides in pig feeds are generally classified into three groups: cellulose, hemicellulose and pectins. This classification is essentially arbitrary and does not fully reflect properties such as degradability. Older analytical methods basically determined fibre as the residue insoluble in a specific solvent. As could be expected, this can lead to widely divergent results. For example the crude fibre method, which recovered most of the cellulose and some of the lignin, gives a content much less than dietary fibre (Table 1). The detergent methods, acid detergent fibre (ADF) and neutral detergent fibre (NDF), give results somewhat intermediate as the former recovers almost all cellulose and lignin and the latter these two components plus most of the hemicellulose (Van Soest, 1982). However, the relationships between these methods are not predictable. Further, as each method recovers a specific fraction, apparent degradability will differ with analytical method employed. As can be seen from Table 2, the amount of degraded fibre in the faeces will vary from 15 to 123 g/kg intake, depending on whether this is calculated on the ADF or dietary fibre method. Thus the estimated energetic value of fibre in the feed would differ over eight fold.

Table 2. Content (g/kg DM) and ileal and faecal apparent degradability (%) of dietary fibre (DF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude fibre (CF) in a cereal-based pig diet.

	DF	NDF	ADF	CF
Dietary content (g/kg DM)	196	163	47	46
Ileal digestibility (%)	23	28	-6	7
Feacal digestibility (%)	63	59	32	36

A complete analysis of the fibre content of feeds gives no information on the physiological attributes of the fibre present, although an experienced analyst can make a few generalized statements. In an effort to further characterize fibre many analytical methods include a seperate determination of soluble fibre. Unfortunately the yield of soluble fibre is very much dependent on the extraction conditions employed, with pre-treatment, temperature and pH of particular interest (Graham et al., 1988). Further, solubility per se does not necessarily confer any particular characteristics to a fibre, even though soluble fibres tend to be more soluble and to have a greater effect on the digestion of other nutrients in the small intestine (Graham, 1988). Certain fibres can also be partially solubilized during passage through the small intestine, with the solubility of NSP residues in a sugar beet pulp based diet increasing from 11% in the diet to 20% at the duodenum and 35% at the ileum (Graham et al., 1986). Little soluble fibre is recovered in the faeces of pigs.

In conclusion, methods for the complete analysis of dietary fibre are available and their use has greatly advanced the understanding of the physiological role of fibre in pigs. However further research is needed into the relationships between fibre structure and physico-chemical properties such as degradability and water- and ion-binding capacities. A better understanding of the processes of fibre degradation and utilization would also be welcome.

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THE INFLUENCE OF SUPPLEMENTARY FEED ENZYMES ON NUTRIENT DISAPPEARENCE AND DIGESTA CHARACTERISTICS IN THE GI-TRACT OF EARLY WEANED PIGS

J. Inborr[1], M.R, Bedford[2], J.F. Patience[2] and H.L. Classen[2]

[1]Finnfeeds International Ltd., 41-51 Brighton Road, Redhill, Surrey RH1 6YS, United Kingdom.
[2]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7K 0I7.

Abstract

Two trials were conducted to investigate the influence of supplementary feed enzymes on the performance and gastro-intestinal function of the weanling pig. In experiment 1 pentosanase and in experiment 2 β-glucanase were added to rye-based or barley-based control diets, respectively, containing chromic oxide as an external marker. After 10 days on the test diets, pigs were euthanized and contents of the small intestine (divided into 4 segments), colon and rectum collected and analysed. Pentosanase supplementation did not directly affect performance or digestibility of starch or protein in any gut segment. However, digesta viscosity was increased ($p<0.05$) in segments 1 and 3 of the small intestine, whereas pH in segment 3 and dry matter in segment 1 were decresed ($p<0.05$) by the enzyme supplement. In Expt. 2, β-glucanase increased weight gain ($p<0.05$) and advanced protein, but not starch, digestion along the GI tract. pH, dry matter and viscosity were unaffected. By altering digesta pH, viscosity and dry matter, pentosanase at the level added appeared to have indirect and possibly undesirable effects on rye digestion. In barley, β-glucanase effects appeared to be more direct and beneficial. Information on specific gastro-intestinal inter-action between endogenous and exogenous enzymes will lead to optimized use in pig production.

Introduction

Due to the slow development in the production of digestive enzymes the capability to digest nutrients (other than those in the sow's milk) is limited in the young pig. For example, the production of maltase increases up to the 8th week of age (Aumaitre & Corring, 1978). In addition, reduced amylase and protease production in the immediate post weaning period have been reported (Lindemann et al., 1986; Owsley et al., 1986), which may impede the digestion of dietary starch and proteins. The digestion of fibre poly-saccharides is even more limited, since fibre degrading enzymes cannot be produced by the pig and the microflora capable of doing so is poorly developed (Graham et al, 1988a).

Rye and barley contain soluble fibre referred to as the pentosans (rye) and β-glucans (barley). In the chick, these are thought to interfere with digestion by conferring viscous

characteristics to the digesta, thereby reducing the physical exposure of substrates to the endogenous enzymes (Fengler & Marquardt, 1988). Addition of microbial enzymes capable of degrading these components have significantly improved the nutritive value of these two cereal grains in the chick (Hesselman, 1983: Pettersson & Åman, 1988). In the pig, enzyme supplementation of rye- and barley-based diets have not given as consistent results as in the chick (Thacker et al., 1988; Graham et al., 1986). The age of the pig may well influence its response to enzyme supplementation as it appears to do in chickens (Säterby, 1985).

The objectives of this study were to use young, weanling pigs in an attempt to determine whether their immature digestive system was further compromised by feeding rye or barley, and if so, whether addition of a pentosanase and β-glucanase preparation to the rye and barley diets, respectively, would alleviate this situation by increasing the rate of digestion. This was determined by measuring digestibility of starch and nitrogen in transit through the small intestine.

Materials and Methods

Pairs of littermate pigs were selected at weaning from a minimum disease herd and assigned to one of the experimental treatments. Non-littermates were housed in pairs in pens, providing 6 pens and 12 pigs per treaement in each of two replicate groups. Following a five day acclimation period, the experimental diets - basal with and without added enzymes (Table 1) - were offered ad libitum for 10 days in a completely randomized design, during which feed intake and body weight change were recorded.

Table 1. Composition of the basal diets (%).[1]

Main ingredients	Experiment 1	Experiment 2
Rye	63.2	0
Hulless barley	0	63.2
Soybean meal (47)	20.4	20.4
Dried skim milk	5.0	5.0
Nutrient content, per kg		
Digestible energy, MJ	13.9	13.5
Crude protein, g	162.1	188.2
Lysine, g	12.0	11.8
Calcium, g	10.0	10.0
Phosphorus, g	8.5	8.5

[1] Enzyme preparation added to basal diet at 2 g/kg at the expense of rye and barley in experiments 1 and 2, respectively.

Following 10 days on the experimental diets, the pigs were euthanized by barbituate overdose administered intravenously,

followed by exsanguination. The abdominal cavity was exposed and the intestinal tract removed. The small intestine was divided into 4 segments of approximately equal length. pH was immediately determined and the results corrected for equal sample temperature. The total contents of each segment were collected into plastic bags and then frozen in liquid nitrogen (Expt. 1) or on dry ice (Expt. 2). Samples of colonic and rectal contents were handled in a similar fashion. For viscosity determination in Expt 1, 0.3 g of sample was resuspended in 1.5 ml water and incubated at 37°C for 30 mins. Viscosity was determined on the 12,000 g supernatant (5'). In Expt. 2, viscosity was determined directly on the 12,000 g (5') supernatant of collected digesta.

Table 2. Effect of enzyme supplementation of a rye-based diet on weight gain, feed intake and feed efficiency in young pigs.

Treatment	Weight gain, kg	Feed intake, kg	Feed/ Gain
Control	4.24	6.94	1.64
Enzyme	4.24	6.64	1.57
p values			
treatment	0.684	0.525	0.753
feed intake	0.104		0.733

Results

Weight gain, feed intake and feed efficiency were unaffected by the pentosanase supplementation (Table 2). Starch and protein digestibility were also unaffected throughout the entire intestinal tract (Table 3), but viscosity increased significantly in sections 1 and 3 and pH decreased in section 3 of the small intestine due to the pentosanase. Dry matter was significantly decreased by enzyme supplementation in section 1.

β-glucanase supplementation significantly improved weight gain, whereas feed intake and efficiency were unaffected (Table 4). Enzyme supplementation of the barley-based diet significantly improved nitrogen digestibility in the colon, but did not influence starch digestibility (Table 5). Viscosity, pH or dry matter in each section of the GI tract were affected by treatment.

Discussion

Enzyme supplementation of both rye and barley-based diets has generally proven less effective and consistent in pigs than in poultry. For example, Newman et al. (1980) did not obtain any improvement in the performance of growing pigs when adding bacterial diastase to a hulless barley-based

diet, but did so in a repeated attempt three years later (Newman et al., 1983). In contrast, consistent improvements in both rate and efficiency of gain due to enzyme supplementation of chicken diets based on both rye and barley have been repeatedly reported (Antoniou & Marquardt 1981; Rotter et al., 1989). In the current trials with weanling pigs, enzyme supplementation of the rye-based diet did not improve animal performance, whereas weight gain was significantly improved on the hulless barley-based diet.

Table 3. Effect of enzyme supplementation of a rye-based diet on starch and nitrogen digestibility, and some digesta parameters in young pigs (treatment means).

Treatment	Sect 1	Sect 2	Sect 3	Sect 4	Colon	Rectum
				Starch		
Control	6.71	32.5	59.8	81.9	94.9	96.5
Enzyme	-2.40	29.9	66.0	84.1	95.5	97.1
p values	0.521	0.652	0.967	0.714	0.516	0.922
				Nitrogen		
Control	-59.	-27.0	27.6	63.4	73.2	75.8
Enzyme	-103.	-33.0	-4.7	57.7	75.9	64.6
p values	0.166	0.238	0.605	0.484	0.262	0.270
				Viscosity		
Control	3.75	4.46	4.38	6.01	4.76	5.28
Enzyme	5.07	4.60	6.03	6.89	4.71	4.85
p values	0.015	0.874	0.018	0.237	0.894	0.431
				pH		
Control	6.04	6.13	6.28	6.51	6.61	6.64
Enzyme	6.28	6.35	6.00	6.57	6.43	6.64
p values	0.280	0.562	0.042	0.695	0.089	0.926
				Dry Matter		
Control	10.31	10.01	10.35	10.19	23.48	24.41
Enzyme	7.75	9.67	8.88	10.21	25.04	26.51
p values	0.024	0.776	0.278	0.992	0.344	0.102

Pentosanase supplementation significantly increased digesta viscosity in segments 1 and 3, which correlated with reduced protein digestibility, suggesting that digestion may be limited by diffusion. Pettersson and Åman (1989) also reported an increase in viscosity of an extract of a rye-

Table 4. Effect of enzyme supplementation of a barley-based diet on weight gain, feed intake and feed efficiency in young pigs.

Treatment	Weight gain, kg	Feed intake, kg	Feed/ Gain
Control	5.24	9.05	1.75
Enzyme	6.14	9.57	1.55
p values			
treatment	0.045	0.251	0.102
feed intake	0.170		0.433

Table 5. Effect of enzyme supplementation of a barley-based diet on starch and nitrogen digestibility, and some digesta parameters in young pigs (treatment means).

Treatment	Sect 1	Sect 2	Sect 3	Sect 4	Colon	Rectum
			Starch			
Control	-42.0	6.1	49.3	73.8	92.8	95.6
Enzyme	-48.0	19.3	49.4	71.5	94.8	96.3
p values	0.201	0.279	0.902	0.394	0.303	0.251
			Nitrogen			
Control	-164.0	-83.0	5.97	63.4	61.0	67.0
Enzyme	-171.0	-18.0	19.40	42.6	71.3	71.5
p values	0.682	0.291	0.412	0.247	0.038	0.192
			Viscosity			
Control	2.89	3.21	3.08	3.17		
Enzyme	2.87	2.97	2.76	3.96		
p values	0.312	0.448	0.212	0.375		
			pH			
Control	5.86	5.87	6.20	6.19	6.46	
Enzyme	5.76	5.87	6.20	6.19	6.34	
p values	0.529	0.988	0.995	0.975	0.331	
			Dry Matter			
Control	12.54	13.11	12.14	12.10	24.54	25.24
Enzyme	11.74	13.23	12.57	12.60	25.62	25.29
p values	0.554	0.920	0.735	0.653	0.375	0.975

based diet when incubated with a high level of pentosanase and concluded this was due to enzyme-mediated release of insoluble pentosans into solution. This may explain the observations made in this trial.

Protein digestibility was significantly improved in the colon and further analysis of sections 2-4 as a whole revealed a significant ($p<0.05$) improvement due to the β-glucanase supplementation of the barley-based diet. Improved nitrogen digestibility has been observed with β-glucanase supplementation of barley-based diets in 19-25 kg (Graham et al., 1988b) and 40 kg pigs (Thacker et al., 1988) when measured at the terminal ileum. In rye-fed pigs, numerically decreased protein digestibility correlated with increased viscosity in sections 1 and 3 ($p=0.028$ and 0.025), indicating that viscosity may influence protein digestion in young pigs fed diets with high rye (pentosan) content. Similar observations were not made in the barley fed pigs.

Starch digestibility was unaffected by the changes in digesta characteristics of both diet types due to the enzyme treatments. This is in variance with previous reports using ileum cannulated pigs (Graham et al., 1988b, 1989) and observations made in chickens (Hesselman, 1983).

In summary, pentosanase supplementation of the rye-based diet did not seem to provide any benefit, whereas a significant improvement in weight gain was brought about by β-glucanase supplementation of the barley-based diet.

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DIGESTION AND UTILIZATION OF D-XYLOSE IN PIGS AS AFFECTED BY AGE, FREQUENCY OF FEEDING AND DIETARY LEVEL

J.B. Schutte, G.M. Beelen, G.B. Derksen and J. Wiebenga.

TNO-Institute of Animal Nutrition and Physiology (ILOB)
P.O. Box 15, 6700 AA Wageningen, The Netherlands.

Abstract

The pentose sugar D-xylose is one of the most abundant components which will result from in a complete chemical or enzymatic hydrolysis of nonstarch polysaccharides of feed ingredients of vegetable origin. Because of the uncertainties about the nutritional value of D-xylose, two trials with pigs were conducted to investigate the urinary excretion of xylose in relation to the age of pigs, frequency of feeding and dietary inclusion level of D-xylose. Moreover the effect of inclusion of D-xylose in pig diets on N and energy utilization was examined.

Urinary excretion of xylose was not significantly affected by age and frequency of feeding. The extent of urinary excretion of xylose in % of intake increased linearly ($P < 0.05$) as the dietary level of this sugar was increased. In pigs fed on a diet containing 25 g D-xylose/kg about 20% of the D-xylose consumed appeared in the urine. This level increased to about 43% when pigs were fed a diet containing 100 g D-xylose/kg. Retention of N was slightly decreased when pigs were fed 100 g D-xylose/kg diet. Urinary excretion of energy bearing components tended to increase in pigs fed on D-xylose diets. Liver and kidney weight, pH of urine and blood composition were not significantly affected by inclusion D-xylose in the diets.

Introduction

Nonstarch polysaccharides (NSP) can form a major fraction of the carbohydrate content of practical diets for pigs. These NSP include a mixture of substances such as cellulose, hemicellulose, pectin and oligosaccharides which contain hexose and pentose sugars and uronic acids. It is well known that NSP are resistant to the digestive enzymes of saliva, stomach and small intestine of pigs. As a result they pass to the hind gut where microbial degradation takes place. The end products of a microbial degradation of NSP (lactic acid, volatile fatty acids) are readily absorbed and can be utilized by the pig as an energy source, but with a lower efficiency than e.g. glucose (Agricultural Research Council, 1981; Just et al., 1983; Van Es, 1987).

In a literature review, Chesson (1987) concluded that the digestibility of feed ingredients containing high levels of NSP can be improved by treatment with enzymes which can hydrolyse the NSP to monosaccharides. This was confirmed in a recently performed study at our institute (Schutte et al., 1990). Our study showed that in addition to an improvement of the digestibility of cell wall components, digestion of protein and fat was also improved in pigs when wheat bran was treated with a cellulolytic enzyme preparation. However, it remains an open question as to what extent pentose sugars and uronic acids can be utilized in pigs. Next to D-glucose the pentose sugar D-xylose is one of the most important components to be released in an enzymatic hydrolysis of NSP (Carre & Brillouet, 1986; Brillouet et al., 1988). It is well recognized that D-xylose is readily absorbed from the intestinal tract

of monogastric animals (Cori, 1925; Miller & Lewis, 1932; Arnal-Peyrot & Adrian, 1974; Schutte et al., 1991a, 1991b). These studies also showed that part of the digested D-xylose is excreted in the urine. The extent of urinary xylose output may be affected by several factors like intestinal bacterial growth, state of health, age and dietary level (Hindmarsh,1976).

In the two trials reported herein the influence of frequency of feeding, age and dietary D-xylose level on urinary excretion of xylose was investigated in pigs. In addition, in these trials the effect of D-xylose on nitrogen and energy utilization was examined.

Materials and Methods

Animals and diets

Two separate trials were conducted with growing castrated male pigs (Dutch Landrace x Dutch Yorkshire). In both trials the pigs were individually housed in metabolism cages under a 12 h light - 12 h twilight cycle throughout. The nutritionally complete basal diet used was based on maize, wheat starch and isolated soya protein. The composition of the basal diet and its chemical characteristics are shown in Table 1.

Table 1. Composition of the basal diet.

Ingredient	g/kg	
Maize meal	287	
Wheat starch	287	
Soyabean oil	40	
Animal fat	40	
Soy isolate (880 g protein/kg)	223.3	
Cellulose ("Arbocel B800")	60	
Monocalcium phosphate	24	
Limestone	10	
Potassium bicarbonate	15	
Iodized salt	10	
Mineral/vitamin mix *	5	
DL-methionine	0.7	
Contents (g/kg)	Expt 1	Expt 2
Dry matter **	886	891
Gross energy (MJ/kg) **	17.6	17.8
Crude protein **	216	223
Calcium ***	8.4	8.4
Phosphorus ***	7.0	7.0
Lysine ***	12.6	12.6
Methionine + cystine ***	6.8	6.8
Threonine ***	8.3	8.3
Tryptophan ***	2.6	2.6

* Provided (mg/kg diet): magnesium, 400; zinc, 110; copper, 25; manganese, 45; iron, 80; cobalt, 0.5; selenium 0.1; thiamin, 2; riboflavin, 5; nicotinamide, 30; pantothenic acid, 12; pyridoxine, 3; cyanocobalamin, 0,04; biotin, 0.1; folic acid, 1; menadione, 3; ascorbic acid, 50; retinol, 3.1; cholecalciferol, 0.045; vitamin E, 40; choline chloride, 1000.
** Analysed *** Calculated

The test sugars (D-glucose and D-xylose), supplied as anhydrous monosaccharides, were substituted by weight for wheat starch.

In both trials the experimental diets were fed at a daily rate of approximately 0.9 MJ metabolizable energy (ME)/kg metabolic body weight, assuming that D-xylose has the same ME content as D-glucose. The daily amount of feed was adjusted weekly according to body weight. The feed was mixed with water (1 part feed + 1 part water). In addition, water was freely available.

Experimental protocol.

Experiment 1. The objectives of this trial were to determine the effect of D-xylose at a dietary inclusion level of 100 g/kg on faecal digestibility of nitrogen (N) and gross energy (GE), retention of N, and urinary excretion of xylose, N and energy in relation to feeding frequency. The feeding frequencies applied were 2 and 4 times/d, respectively. The daily amount of feed was offered at two, respectively, four equal meals at intervals of 12 and 6 h, respectively.

The trial involved 12 pigs with a mean age of 8 weeks at the start of the trial. The pigs were accustomed to cages and basal diet (Table 1) for 25 days before starting the experimental period. At the start of the experimental period three groups of four pigs, each of similar average body weight, were formed and fed diets containing either D-glucose or D-xylose. As is illustrated in Table 2, the experimental period consisted of two phases. During both phases the D-glucose diet (treatment 1) was fed four times/d, whereas the frequency of feeding of pigs fed the D-xylose diets (treatments 2 and 3) was changed in phase two. Each of the two phases consisted of a 4 d adaptation and a 5 d collection period.

At the start of the experimental period, the pigs weighed 25.2 (SD 1.1) kg and at the end 34.6 (SD 1.2)kg.

Table 2. Design of experiment 1.

Treatment	n	Sugar*	Frequency of feeding	
			Phase 1	Phase 2
1	4	D-glucose	4 times/d	4 times/d
2	4	D-xylose	2 times/d	4 times/d
3	4	D-xylose	4 times/d	2 times/d

* Included in the diet at a level of 100 g/kg.

Experiment 2. In this trial the effect of graded dietary levels (25 to 100 g/kg) of D-xylose on N digestibility, N retention, and urinary excretion of xylose and N in relation to the age of the pigs was examined. Moreover, specific density and pH of urine, liver and kidney weight, and blood composition were examined.

This trial involved 16 pigs: 8 young pigs with an age of 7 weeks and 8 older pigs with an age of 15 weeks at the start of the trial. The pigs were accustomed to cages and basal diet (Table 1) for 14 days before starting the experimental period. At the start of the experimental period two treatment groups each involving 4 young and 4 older pigs were formed and fed diets containing either D-glucose or D-xylose. As is

illustrated in Table 3, the experimental period consisted of four phases, during which time the pigs of the two treatment groups were fed consecutively on a diet containing 25, 50, 75 and 100 g D-glucose or D-xylose /kg. The daily amount of feed was offered at two equal meals at intervals of 12 h. Each of the four phases consisted of a 3 d adaptation and a 4 d collection period. At the start of the experimental period the young and older pigs weighed on average 17.0 (SD 1.3) and 55.3 (SD 5.6) kg, respectively. At the end of this period the pigs weighed 27.9 (SD 1.8) and 74.8 (SD 8.0) kg, respectively.

Table 3. Design of experiment 2.

Treatment	n	Sugar	Dietary sugar level (g/kg)			
			phase 1	phase 2	phase 3	phase 4
1	8	D-glucose	25	50	75	100
2	8	D-xylose	25	50	75	100

Faeces and urine collection

Faeces were collected directly into a bag fitted around the anus, and the urine was collected using a funnel fitted under the cage. Total collection of faeces and urine was carried out during each five (Expt 1) and four (Expt 2) 24 h collection periods from the individual animals at intervals of 12 h. The faeces were stored at -20 °C. All faeces produced during each collection period were pooled for each pig separately, homogenized and sampled. Next the samples were freeze-dried. Urine was collected in containers provided with merthiolate sodium (Thimerosal, BDH Chemicals Ltd, Poole, England) at intervals of approximately 4 h. The portion from each interval was pooled daily from individual animals. A representative sample of 10 % of the pooled urine was taken and frozen at -20 °C. The five (Expt 1) and four (Expt 2) day sub-samples of urine were pooled for each animal separately, homogenized and sampled. Faeces and urine were kept at -20 °C between sampling and before analysis.

Analytical methods

Samples of feed and freeze-dried faeces were milled to pass through a 1.0 mm screen (Retsch mill ZMI, Retsch BV, Ochten, The Netherlands) before analysis. All analyses were carried out in duplicate. Dry matter was determined by drying the samples to constant weight at 101 °C. Inorganic matter and nitrogen were determined by standard methods (Association of Official Analytical Chemists, 1975). Gross energy was determined using an IKA-C 4000 adiabatic bomb calorimeter.
Concentrations of glucose and xylose in urine were determined according the procedure described by Schutte et al. (1991a).

Statistical analysis

The results of both trials were analysed by means of analysis of variance (Cochran & Cox, 1957). The computer program Genstat 5 (Reference Manual, 1987, Oxford University Press, New York) was used to calculate the analysis of variance. Although the treatments were confounded by time and age, it was assumed that differences are due to

the test sugars or increase in dietary sugar. In Expt 1 the treatment factors were type of sugar, frequency of feeding and phase. The differences in results achieved on the D-xylose diets in the first and second phase at an equal feeding frequency were small and statistically not significant. Therefore, the results of phase 1 and 2 of pigs fed the D-xylose diet two times/d and those obtained at feeding this diet 4 times/d were combined in the statistical analysis. In Expt 2 the treatment factors were type of sugar, dietary level of sugar and age. In this experiment the sum of squares for levels was partitioned into a set of orthogonal linear and quadratic polynomial regression components.

Results and Discussion

Table 4, which incorporates values of a previous study (Schutte et al., 1991a), indicates that D-xylose was digested almost completely at the terminal ileum. This would suggest an almost complete absorption of this pentose sugar as such. However, administration of D-xylose to pigs was associated with an increased ileal flow of VFA and a decreased ileal chyme pH. Both symptoms point to a more extensive microbial activity in the small intestine. This is further supported by the decrease in apparent ileal digestibility of N in pigs fed on the D-xylose diets. From the results of this study is was concluded that at least part of the ingested D-xylose has been consumed by the intestinal microbes. This conclusion is supported by data of Schiffer et al. (1962), Cooke et al. (1963) and Goldstein et al. (1970) who reported an increased urinary xylose excretion after antibiotics in man with small intestinal diverticulosis. The extent of microbial degradation of D-xylose in our study with pigs cannot be derived simply from the differences in ileal flow of VFA between the D-glucose and D-xylose treatments, because some of the VFA will be absorbed already in the small intestine. Wyngaarden et al. (1957) reported that approximately 15% of an intravenously infused dose

Table 4. Apparent ileal digestibility data of pigs fed on D-glucose and D-xylose diets *.

Sugar	D-glucose	D-xylose	
Dietary level (g/kg)	200	100	200
Digestibilities			
OM	87.6^{a}	87.2^{a}	84.7^{b}
GE	87.6^{a}	87.5^{a}	84.5^{b}
N	90.3^{a}	89.1^{a}	87.2^{b}
D-glucose	99.3	-	-
D-xylose	-	98.7^{a}	98.6^{a}
Ileal chyme pH	6.5^{a}	6.2^{b}	6.0^{c}
Ileal flow of VFA (mg/12h)	1106^{a}	2062^{b}	4888^{c}

* Data from Schutte et al. (1991a).

^{a,b,c}Within a row, mean values with different superscript letters were significantly different ($P < 0.05$).

of D-xylose can be recovered as carbon dioxide in the expired air. In a previous study (Schutte et al., 1991a) it was found that at a dietary inclusion level of 100 g/kg, about 45% of the ingested D-xylose was excreted in the urine of pigs. Assuming that 15% of the dose has been metabolized to carbon dioxide (Wyngaarden et al. (1957), the remaining 40% not accounted for may have been fermented by the intestinal microbes.

One of the main objectives of the present experiments was to investigate whether or not urinary excretion of xylose is affected by feeding frequency, age and dietary inclusion level of this pentose sugar in pigs.

Table 5. Expt 1. Influence of feeding frequency of D-xylose diets on N and energy utilization, and urinary excretion of xylose.

Sugar(100g/kg diet)	D-glucose	D-xylose		SED	
Frequency of feeding	4 times/d (1)	4 times/d (2)	2 times/d (3)	1~2 1~3	2~3
DM intake (g/pig/d)	754[a]	751[a]	764[a]	9.9	4.1
Faecal digestibility (%)					
N	92.5[a]	92.9[a]	93.0[a]	0.5	0.4
Gross energy	90.3[a]	90.5[a]	ND	0.6	-
Urinary excretion (% of intake)					
Glucose	+	+	+	-	-
Xylose	+	41.0[a]	39.9[a]	-	1.8
N	32.2[a]	35.9[a]	37.4[a]	2.4	2.3
Energy	2.3[a]	7.2[b]	ND	0.6	-
N retained (% of intake)	60.4[a]	57.0[a]	55.6[a]	2.3	2.2
ME (MJ/kg DM)					
Diets *	16.7[a]	15.9[b]	ND	0.1	-
D-glucose **	15.2	-	-	-	-
D-xylose ***	-	7.8	ND	-	-

SED = standard error of difference of means
ND = not determined
\+ Small traces of glucose (0.1-0.3 g/L) and xylose (0.01-0.2 g/L) were found in the urine of these experimental treatments.
* Corrected to zero N balance by using the factor 31.4 kJ/g retained N.
** Calculated as 98% of GE value.
*** Derived from the differences in ME value between the D-glucose and D-xylose diets.
a,b Within a row, mean values with different superscript letters were significantly different (P<0,05).

The results of Expt 1 (Table 5) show that urinary excretion of xylose was not clearly affected by the frequency of feeding diets containing this pentose sugar. The dietary inclusion level of D-xylose, however, did appear to effect the urinary excretion of xylose (Expt 2, Table 6). In both young and older pigs a positive correlation between the dietary level and the urinary excretion of xylose was found. This correlation was more pronounced in the young than in the older pigs. Taking the results of both ages together it appeared that urinary excretion of xylose in % of intake was linearly ($P<0.01$) increased when the dietary level of this sugar was increased. Similar results were found in a previous trial with ileostomised adult cocks (Schutte et al., 1991b). Wagh & Waibel (1966) reported that the ME value of D-xylose in chicks was decreased when the dietary level was increased. This observation suggests also a dosage-dependent urinary excretion of D-xylose. In the present study no VFA measurements in the ileal chyme were performed. Thus it cannot be simply stated that the observed dosage-dependent urinary excretion of xylose is exclusively due to differences in microbial degradation of this sugar at the different dietary levels. In addition the low renal threshold for this sugar as suggested by Loos (1954) may have affected our results for urinary excretion.

Fowler & Cooke (1960), Finlay et al. (1964) and Hindmarsh (1976) reported that urinary xylose output in man declines with age. The reason for this is unknown, but it has been postulated that renal function, and consequently xylose excretion, is affected by the ageing process (Kendall, 1970). In our study an age dependent urinary excretion of xylose was not clearly demonstrated (Table 6).

Table 6. Expt 2. Urinary excretion of xylose and energy in young (A) and older (B) pigs fed on D-glucose (Gluc) and D-xylose(Xyl) diets.

Sugar	Dietary level (g/kg)	DM intake (g/pig/d)		Xylose excreted in urine (% of intake)		Energy excreted in urine (% of intake)	
		A	B	A	B	A	B
Gluc	25	524	1142	+	+	2.76	3.61
	50	569	1220	+	+	2.92	3.36
	75	621	1291	+	+	2.75	3.41
	100	678	1362	+	+	2.96	3.34
Xyl	25	540	1100	18.4	22.6	3.87	4.34
	50	604	1254	29.2	34.1	5.04	5.96
	75	666	1326	36.6	41.3	5.51	7.60
	100	723	1435	43.2	42.5	8.18	9.19

Analysis of variance (exclusive DM intake)

Source of variation between animal stratum	Probability	
Age	0.41	0.01
Sugar	-	0.01
Age x sugar	-	0.14
Residual MS	110.5	0.46

+ Small traces (0.01-0.1 g/l) of xylose were found in the urine of these experimental treatments.

Table 7. Expt 2. Apparent faecal digestibility of N, urinary excretion of N and N retained in young (A) and older (B) pigs fed on D-glucose (Gluc) and D-xylose (Xyl) diets.

Sugar	Dietary level (g/kg)	N digestibility (%)		N excreted in urine (% of intake)		N retained (% of intake)	
		A	B	A	B	A	B
Gluc	50	92.8	93.9	38.1	51.5	54.7	42.4
	100	93.2	94.3	40.4	53.7	52.8	40.7
Xyl	50	93.0	94.3	38.5	51.4	54.5	42.9
	100	93.1	94.4	41.6	55.9	51.5	38.5

Analysis of variance

Source of variation between animal stratum	Probability		
Age	0.01	0.01	0.01
Sugar	0.65	0.60	0.65
Age x sugar	0.73	0.95	0.99
Residual MS	1.08	22.81	23.0

Table 8. Expt 2. Specific density and pH of urine, and liver and kidney weight (in % of body weight) in young (A) and older (B) pigs fed on D-glucose (Gluc) and D-xylose (Xyl) diets.

Sugar	Urine values *				Organ weights			
	Spec. density		pH		Liver		Kidney	
	A	B	A	B	A	B	A	B
Gluc	1.006	1.023	7.1	6.7	1.8	1.5	0.372	0.282
Xyl	1.014	1.026	7.0	6.6	1.9	1.6	0.352	0.301

Analysis of variance

Source of variation between animal stratum				
Age	0.01	0.01	0.01	0.01
Sugar	0.06	0.10	0.34	0.96
Age x sugar	0.34	0.75	0.56	0.22
Residual MS	32.11	0.01	0.02	0.01

* Determined during phase 4 only; each value represents 16 individual observations (one observation/animal/d).

The losses of xylose into the urine were reflected in the urinary excretion of energy (Tables 5 and 6), but the differences in urinary excretion of energy between the D-glucose and D-xylose treatments could not be fully explained by the xylose losses. Calculations have pointed out that when the increases in urinary excretion of energy of the D-xylose treatments over the D-glucose treatments were attributed to D-xylose, this would represent about 50% of the D-xylose intake in Expt 1 (Table 5). Thus compared to the losses of xylose in the urine (40%), 10% of the energy excreted in the urine is not accounted for. In Expt 2 (Table 6) the extra losses of energy into the urine of pigs fed on the D-xylose diets would represent about 47 and 52% of the xylose intake in young and older pigs, respectively. The values found for urinary excretion of xylose in both young and older pigs were much lower, being as a mean 32 and 35%, respectively. It is obvious that other energy bearing substances than xylose have contributed to the extra losses of energy into the urine of pigs fed on the D-xylose diets. This is supported by the slight increase in urinary excretion of N when pigs were fed on diets containing 100 g D-xylose/kg (Tables 5 and 7). Wise et al. (1954) reported that N retention was significantly decreased when pigs were fed on a diet containing 560 g D-xylose/kg, indicating also a greater urinary excretion of N. These investigators believe that the observed reduction in N retention in pigs fed on D-xylose diets was due to an energy deficiency and consequently greater N catabolism. This is supported by data of Wagh & Waibel (1966) who found that plasma uric acid was significantly increased when chicks were fed on diets containing 200 and 400 g D-xylose/kg. Further they reported that feeding these diets to birds resulted in decreased liver weights. The data of Wise et al. (1954) and Wagh & Waibel (1966) are not strictly comparable with the results of our study since lower levels of D-xylose were included in our diets. An energy deficiency when occuring in our study, was not reflected in liver weights (Table 8).

Wagh & Waibel (1967) reported that blood hematocrit, cholesterol, serine and proline in plasma were increased and plasma glutamic acid was decreased when birds were fed on diets containing 100 to 400 g D-xylose per kg. In order to study whether or not inclusion of D-xylose in pig diets will change blood composition, blood samples were collected from pigs of Expt 2. These samples were taken after termination of the last phase (phase 4) and involved the following determinations; leucocytes, hemoglobin (Hb), erythrocytes, hematocrit, mean corpuscular value, mean corpuscular Hb concentration, glucose, bilirubin, bilirubinester, cholesterol, triglycerides, albumine and total protein. The differences in these blood parameters between pigs fed on the D-glucose diets and those fed on the D-xylose were small and not significant. Further it is noteworthy that histo-pathological examination of the liver and kidneys did not show abnormalities in pigs fed on either D-glucose or D-xylose diets (Expt 2). Specific density of urine appeared to be slightly increased (Table 8) when pigs were fed on diets containing D-xylose; this will be the result of the increased urinary excretion of energy on this diet. No effect of D-xylose on the pH value of urine was observed (Table 8).

In conclusion it can be stated that the energy value of D-xylose is much lower than that of D-glucose. From the results of Expt 1 it was calculated that at a dietary level of 100 g/kg, D-xylose has a ME value of only 7.8 MJ/kg, which value is approximately 50% of that of D-glucose. Considering the data for urinary excretion of xylose, it may

be expected that at lower dietary inclusion levels than 100 g/kg the ME value of this pentose sugar will increase. The net utilization of D-xylose in pigs is difficult to access from the present study. This because of the unknown metabolic pathway of this sugar. The results of a previous study (Schutte et al., 1991a) indicated that at least part of the xylose is fermented in the small intestine. It may even be possible that all xylose has to be fermented before it can be used as an energy source for pigs.

Further studies are in preparation to clarify the metabolism of this sugar in pigs.

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EFFECTS OF CARBOHYDRATE SOURCES AND ORGANIC ACIDS ON INTESTINAL MICROFLORA AND PERFORMANCE OF THE WEANLING PIG[1]

A. L. Sutton, A. G. Mathew, A. B. Scheidt, J. A. Patterson, and D. T. Kelly

Department of Animal Sciences and Department of Veterinary Clinical Sciences,
Purdue University, West Lafayette, Indiana, USA 47907

Abstract

Two four week feeding experiments were conducted to determine the effects of carbohydrate sources and organic acids on E. coli and Lactobacillus populations and fermentation patterns in the GI tract of the weanling pig. In Experiment I, 48 weanling pigs were randomly assigned to 6 diets, which included a basal (22% CP) corn-soy control with 10% whey (C), diets with 20% whey (W), 15% corn bran (B), .25% Luprosil-NC® (L), .3% sodium propionate (SP), and .3% sodium fumarate (SF). In Experiment II, 24 weanling pigs were assigned to 4 diets which included the corn-soy based control (C), diets with 15% wheat midds (WM), 5% soy flour (S) and 1% fumaric acid (F). At the end of the trial, stomach, small intestine, cecal and colon digesta were sampled and E. coli, Lactobacillus, and volatile fatty acid concentrations are reported.

E. coli concentrations in the cecum and colon of pigs fed Luprosil-NC® and control were lower compared to other treatments. Sodium fumarate fed pigs had increased ($P<.01$) E. coli concentrations in the duodenum with the same trend in the cecum and colon. Lactobacillus concentrations in the duodenum, cecum and proximal colon increased ($P<.03$) with whey and decreased with sodium fumarate. Incidences of diarrhea were higher with pigs fed the control, whey and sodium fumarate diets. The pigs fed the control and wheat midds diets had higher ($P<.05$) populations of E. coli in the stomach than those fed fumaric acid and soy flour. The pigs fed the soy flour also had lower ($P<.05$) concentrations of Lactobacillus in stomach contents.

Introduction

Digestive and enteric disorders, such as colibacillosis and diarrhea, significantly reduce pig gains and often cause mortality, especially during the stressful time of weaning. The lag phase which often accompanies such disorders causes swine producers unneeded feed and fuel costs, ties up facilities and interrupts the flow of production.

Antibiotic administration on the subclinical level has been utilized to minimize the potential for colibacillosis and therapeutic levels are sometimes used to correct an outbreak. However, there is concern about repeated use of drugs in production units. In addition to cost, other concerns include the development of resistance to the drug by the bacteria, and unintentional contamination of other feeds with drugs requiring long withdrawal periods. In the future, other methods may need to be developed to decrease undesirable enteric bacteria in the gastrointestinal tract of young pigs. This may include the manipulation of the diet to produce an environment which is less favorable to those bacteria capable of causing digestive disorders.

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Establishing large populations of Lactobacilli in the gastrointestinal tract can mediate the adverse effects of E. coli induced diarrhea and other intestinal disorders (Muralidhara, et al. 1977; Nekrep, et al., 1976). Pollman et al., (1980) showed that the addition of lactose with Lactobacillus acidophilus greatly enhanced colonization of L. acidophilus in the intestinal tract and improved weight gains (11%) and feed conversion (1.5%) in young pigs. Use of whey as a lactose source improved energy utilization (Fevrier, 1978) and increased lactase activity in the cecum and colon of pigs and increased volatile fatty acid (VFA) production in the intestine (Kim, et al., 1978). They proposed that increased VFA production contributed significantly to the energy supply of the pig. Others have suggested that VFA and pH have antibacterial effects on E. coli and T. hyodysenteriae.

Addition of organic acids and certain carbohydrate sources have been proposed to alter pH and VFA's of the GI contents in the pig. The objective of this research was to evaluate microflora and fermentation patterns in the weanling pig with different dietary components.

Materials and Methods

Two trials with 4 week old weanling pigs were conducted. In the first trial, 48 pigs, initially weighing 8.3 kg., were randomly assigned to 6 experimental diets with 8 pigs per dietary treatment. All experimental diets were corn-soy based with 22% crude protein and 1.0% lysine but with different carbohydrate or organic acid sources. In addition to the basal corn-soy control (C) with 10% whey, diets with 20% whey (W), 15% corn bran (B), .25% Luprosil-NC® (L), .3% of sodium propionate (SP) and .3% sodium fumarate (SF) were compared. The control diet contained .125%. tylan-sulfa whereas, the other diets did not contain any antibiotic. At weaning, the pigs were placed in individual pens for the 4 week nursery feeding trial. Subsequently, the pigs were euthanatized and stomach, duodenal, cecal and colon (proximal and distal) contents were obtained. Gastrointestinal contents were assayed for Lactobacilli and E. coli, volatile fatty acids, pH, dry matter and ammonium nitrogen. Microbial and VFA data are presented here.

In a second trial, 24 weanling pigs averaging 9.1 kg. were randomly assigned to 4 experimental diets with 6 pigs per dietary treatment. All diets were corn-soy based as indicated in the first study except there was no antibiotic included in the control diet. In addition to the control (C), diets with 15% wheat midds (WM), 5% soy flour [78% crude protein] (S) and 1% fumaric acid (F) were compared. The same protocol was used in this trial as indicated in the first trial.

Results and Discussion

Experiment 1

Although pigs fed Luprosil-NC®, sodium fumarate and whey tended to gain faster and more efficiently than the other treatments, these differences were not statistically significant at the 5% level. Pigs fed sodium propionate and corn bran showed the poorest gains throughout the study. Lowered consumption appeared to affect gains of pigs fed the sodium propionate. Pigs on corn bran gained less due to less efficient conversion of feed nutrients into liveweight gain.

VFA concentrations in intestinal contents were not significantly changed due to diet (Table 1) except for acetate levels in the colon . Pigs fed Luprosil-NC® had the lowest concentrations of acetate in the colon and pigs fed additional dried whey had highest concentrations of acetate in the colon contents. Acetate levels in colon contents were intermediate in pigs fed the other experimental diets. When the individual VFA data were expressed as a percentage of the total VFA production, VFA patterns were not changed due to diets except in the stomach. Pigs fed corn bran had a higher proportion (percentage) of acetate accompanied by a shift to a lower proportion (percentage) of propionate in the total VFA concentrations as compared to the other dietary treatments.

Table 1. Effect of Carbohydrate Sources on Volatile Fatty Acids Concentrations in Pig Intestinal Contents (Exp. 1).

	Volatile Fatty Acid (mM/L)*			
Location/Diet	A	P	B	T
Stomach				
Control	4.68	2.46	2.01	9.30
Corn Bran	11.51	4.80	3.30	22.31
Sodium Fumarate	11.23	10.66	4.46	29.00
Luprosil-NC®	10.23	6.79	4.09	23.42
Sodium Propionate	7.75	6.11	2.11	17.55
Dried Whey	7.67	4.23	2.67	16.50
Duodenum				
Control	1.49	.65	.38	3.13
Corn Bran	1.18	.46	.32	2.41
Sodium Fumarate	4.03	2.56	.84	8.50
Luprosil-NC®	1.35	.45	.45	3.04
Sodiium Propionate	1.75	1.24	.36	3.82
Dried Whey	1.34	.44	.31	2.82
Cecum				
Control	66.68	49.14	26.56	161.79
Corn Bran	63.98	38.73	24.50	135.08
Sodium Fumarate	82.35	53.81	25.59	171.25
Luprosil-NC®	82.71	41.95	28.86	162.96
Sodium Propionate	67.05	43.77	25.40	146.04
Dried Whey	81.57	51.82	29.76	172.61
Colon				
Control	74.06^{ab}	41.35	22.60	149.54^{ab}
Corn Bran	67.81^{ab}	40.01	24.47	142.44^{ab}
Sodium Fumarate	74.45^{ab}	43.48	23.97	153.30^{ab}
Luprosil-NC®	61.28^{b}	33.99	22.69	128.27^{b}
Sodium Propionate	74.98^{ab}	41.95	26.41	155.22^{ab}
Dried Whey	93.84^{a}	46.45	27.39	178.59^{a}

* A = Acetate, P = Propionate, B = Butyrate, T = Total
Means with different superscripts are significantly different ($P<.05$)

Lactobacillus populations tended to be lower in the large intestine of pigs fed corn bran and sodium fumarate diets (Table 2). Conversely, these pigs also had the highest population of E. coli in the cecum and large intestine. Pigs fed whey, Luprosil-NC® and sodium propionate tended to have higher levels of Lactobacillus in the contents of the lower portion of the intestinal system.

Table 2: Effect of Carbohydrates on E. Coli and Lactobacillus (Exp 1).

Diet	Location* S	D	C	PC	DC
	E. coli, Log $_{10}$/gram				
Control	5.619	5.926	6.705	7.079	7.356
Corn Bran	6.033	5.423	6.700	7.160	7.166
Na Fumarate	5.730	6.291	6.961	7.321	7.254
Lurposil-NC®	6.053	6.133	6.589	6.750	6.380
Na Propionate	5.912	5.765	6.756	6.991	6.976
Dried Whey	5.863	5.971	6.792	7.100	6.804
	Lactobacillus, Log $_{10}$/gram				
Control	6.739	7.137	8.501	8.893	8.868
Corn Bran	6.830	7.046	8.459	8.678	8.529
Na Fumarate	6.765	6.478	8.250	8.583	8.759
Luprosil-NC®	7.474	6.683	8.499	8.764	8.727
Na Propionate	7.130	6.983	8.778	9.046	9.150
Dried Whey	6.598	7.091	8.784	9.199	9.021

* S= Stomach, D=Duodenum, C=Cecum, PC= Proximal Colon, DC=Distal Colon

Experiment 2

In the second experiment, pig performance was not changed significantly due to experimental diets. In the first week, the pigs fed the fumaric acid diet tended to gain faster, whereas, pigs fed the control showed the poorest gains and efficiency.

A significantly higher population of E. coli in the stomach of pigs fed control and wheat midds was observed compared to those fed fumaric acid and soy flour (Table 3). Soy flour fed pigs also had lower concentrations of Lactobacillus in pig stomach contents. Diets did not affect the concentration of microbes assayed in the small intestine or cecal and colon samples of the pigs.

Volatile fatty acid concentrations were significantly different between diets in the duodenum and cecum (Table 4). The pigs fed fumaric acid had higher levels of acetate in the duodenum than the pigs fed soy flour and wheat midds. Pigs fed fumaric acid also had higher total VFA than the pigs fed soy flour. In the cecum, the pigs fed the control diet had the lowest levels of acetate and propionate, while the pigs fed fumaric acid had the highest levels of VFA's. The pigs fed the control diet also had significantly lower total VFA's than the other three diets.

Table 3: Effect of Carbohydrate Sources on E. Coli and Lactobacillus (Exp 2)

Diet	Location* S	D	C	PC	DC
		E. Coli, Log_{10}/gram			
Control	4.460[a]	4.887	6.489	6.840	6.737
Wheat midds	4.436[a]	5.067	6.512	6.409	6.415
Fumaric acid	3.870[b]	5.888	6.535	6.724	6.889
Soy flour	3.748[b]	5.360	6.521	6.542	6.707
		Lactobacillus, Log_{10}/gram			
Control	7.703[a]	7.534	8.384	8.314	8.255
Wheat midds	7.402[a]	7.173	9.061	8.067	7.680
Fumaric acid	7.276[a]	7.066	7.978	8.203	8.347
Soy flour	6.686[b]	6.855	7.882	7.735	7.716

* S=Stomach, D=Duodenum, C=Cecum, PC=Proximal Colon, DC=Distal Colon
Means with different superscripts are significantly different (P<.05)

Table 4. Effect of Carbohydrate Sources on Volatile Fatty Acid Concentrations in Pig Intestinal Contents (Exp. 2).

Location/Diet	Volatile Fatty Acid (mM/L)* A	P	B	V	T
Stomach					
Control	8.42	2.85	1.05	.35	12.93
Soy Flour	11.48	5.36	1.96	.52	19.71
Wheat Midds	7.51	3.88	1.92	.68	14.64
Fumaric Acid	10.52	6.30	2.91	.87	20.99
Duodenum					
Control	1.89[ab]	.58	.17	.03	3.05[ab]
Soy Flour	.99[b]	.48	.08	.01	2.13[b]
Wheat Midds	1.20[b]	.63	.19	.04	2.76[ab]
Fumaric Acid	2.79[a]	.67	.10	.04	4.20[a]
Cecum					
Control	52.28[b]	36.34[b]	26.31	7.69	123.56[b]
Soy flour	56.23[b]	54.81[ab]	29.68	10.26	151.07[a]
Wheat Midds	74.36[a]	54.54[ab]	24.92	6.34	161.67[a]
Fumaric Acid	68.29[a]	60.93[a]	27.66	8.06	166.34[a]
Colon					
Control	61.14	39.56	25.03	8.15	135.95
Soy Flour	62.62	44.74	24.71	8.49	143.35
Wheat Midds	73.77	45.02	24.98	6.85	153.09
Fumaric Acid	64.17	42.35	23.18	6.59	139.51

* A = Acetate, P = Propionate, B = Butyrate, V = Valerate, T = Total
Means with different superscripts are significantly different (P<.05)

Conclusions

These studies indicate that carbohydrate sources and specific organic compounds do change enteric bacterial populations and the microbial ecology in different segments of the young pig's intestinal system. Difference in VFA's in the intestinal system also reflected these changes. Fibrous types of carbohydrates, such as corn bran and wheat midds, which require more extensive fermentative pathways of degradation, appeared to lead to higher concentrations of E. coli and lower concentrations of Lactobacillus in the lower gastrointestinal tract.

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BREAKDOWN OF PLANT POLYSACCHARIDES IN THE GASTROINTESTINAL TRACT OF PIGS

Knud Erik Bach Knudsen

National Institute of Animal Science, Animal Physiology and Biochemistry, Foulum, P.O. Box 39, DK-8830 Tjele, Denmark

Abstract

Present investigation was undertaken to study the quantitative breakdown of wheat and oat polysaccharides in the gastrointestinal (GI) tract of pigs. Starch from both cereal grains was almost completely digested (>97%) at the end of the small intestine (ileum), while the recovery of wheat non-starch polysaccharides at this site was 90-97 % of intake. The recovery of oat NSP was significantly lower; 64-82 %, primarily due to a low recovery of mixed linked ß(1->3;1->4)-D-glucans (ß-glucans) in the small intestine. The general order of breakdown of the various NSP constituents over the entire GI tract was ß-glucans>>arabinoxylans>cellulose. While only trace levels of ß-glucans survived breakdown irrespective source, the degree of cell wall lignification played a major role for the breakdown of cellulose and arabinoxylans.

Introduction

Plant polysaccharides, starch and non-starch polysaccharides (NSP), are the most important energy sources when feeding pigs and other single-stomached animals (Just et al. 1983). It is generally accepted that cereal starches represent a highly digestible energy source. Most studies have shown that less than 5% of dietary starch escape digestion in the small intestine of pigs (e.g. Graham et al. 1986a; Bach Knudsen & Hansen, 1990). In contrast to starch no enzymes are present in the small intestine which can cleave the bondings in NSP. Studies with pigs have shown that more than 80% of NSP are recovered at the terminal ileum, while a variable fraction of NSP is broken down by the microflora in the large intestine (e.g. Graham et al. 1986a; Bach Knudsen & Hansen, 1990). The physico-chemical properties of soluble and insoluble dietary fibres (DF: non-starch polysaccharides + lignin), however, suggest that they may affect the digestion and absorption processes in the different segments of the gastrointestinal (GI) tract to a variable degree. Wheat bran for instance has a high proportion of of insoluble DF (Selvandran, 1984) and behave more or less like a inert marker in the GI tract. Consequently, wheat bran has little or no effect on digestion and absorption of nutrients in the small intestine and is highly resistant to microbial degradation in the large intestine of monogastricts. In contrast to the highly insoluble wheat bran, soluble DF as in oat bran and oat products derived from oat endosperm, may increase the viscosity of the luminal content within the GI tract, so delaying gastric emptying, increasing mouth-to-caecum transit time and reducing the rate of nutrient absorption from the small intestine (e.g. Jenkins et al. 1978). Like other soluble DF sources oat fibres are likely to be readily fermented by colonic microorganisms.

The aim of the present investigation was to study the breakdown of polysaccharides from wheat and oats in the various segments of the GI tract of pigs. To a DF depleted wheat flour diet was added DF in the form of either insoluble DF (wheat bran) or soluble DF (oat bran and oat products).

Materials and Methods

The experimental diets were prepared from refined wheat flour (WF) (low DF, control), wheat flour + wheat bran (WFWB), wheat flour + oat bran (WFOB), oat flour (OF), rolled oats + oat bran (ROOB) and oat bran (OB). The six experimental diets were tested in two series of experiments. Expr. 1 include the diets: WF, WFWB, WFOB, and ROOB, while Expr. 2 comprise the diets: OF and OB. The low DF control provided in the balance period 54 g DF/d, diet WFWB 95 g DF/d, diet WFOB 95 g DF/d, diet OF 90 g DF/d, diet ROOB 173 g DF/d and diet OB 235 g DF/d. Pigs (40-50 kg), cannulated at the end of the small intestine were used. Surgery was performed at 30-35 kg and a "T" cannula was placed in the ileum approximately 150 mm anterior to the ileo-caecal junction. The experimental diets were fed to a total of twentyfour ileum cannulated pigs; sixteen pigs in Expr. 1 and eight pigs in Expr. 2 with four pigs on each diets. The animals were fed three times daily at 07.00, 15.00 and 23.00 hours with 1.5-1.8 kg/d adjusted to give the same amount of net energy per day. The feed was thoroughly mixed with water before feeding. After a 7 d adaptation period, faeces were collected quantitatively on dayes 7-11 and ileal digesta on dayes 12-14. Ileal digesta were collected for a total period of 12 hours; on day 12 at 9.00-11.00 hours and 13.00-15.00 hours, on day 13 at 8.00-10.00 hours and 12.00-14.00 hours and on day 14 at 7.00-9.00 hours and 11.00-13.00 hours. The whole procedure was repeted with the same pig on dayes 15-28. After finishing the balance experiment, the pigs were fed the morning ration and killed 4 h post feeding. Immediately after slaughtering, the GI tract was removed and separated by ligatures into twelve sections and samples taken for analysis (Bach Knudsen & Jensen, 1991).

Cr_2O_3 analysis was performed on wet materials, all other analyses were carried out on freeze-dried materials. Cr_2O_3 was determined using the method of Schürch et al. (1950), starch by the enzymic method of Bach Knudsen et al. (1987) and ß-glucans by the fluorometric method of Jørgensen & Aastrup (1987) and the enzymic method of McCleary & Glennie-Holmes (1985). Total, soluble and insoluble NSP and their constituent sugars were determined as alditol acetates by gas-liquid chromatography for neutral sugars, and by a decarboxylation method for uronic acids (A.U.) using a modification of the Theander & Åman (1979) and Theander & Westerlund (1986) and Englyst et al. (1982) procedures. Klason lignin was measured gravimetrically as the residue resistant to 12 M-H_2SO_4 (Theander & Westerlund, 1986). The content of polysaccharide residues was calculated as anhydrosugars, and all digestibilities were calculated relative to the Cr_2O_3 content.

Results and Discussion

The wheat and oat fractions used in the current experiment with pigs varied significantly in chemical and structurel composition. The refined wheat and oat flour were nearly pure endosperm tissues as judge from the high content (g/kg dry matter) of starch of 829 g/kg and 712 g/kg and low content of NSP (34 g/kg and 48 g/kg) and Klason lignin (1 g/kg and 7 g/kg) in wheat and oat, respectively. Notisable for the oat flour is the much higher content of ß-glucans of 22 g/kg in oat flour compared to 4 g/kg in wheat flour. Arabinoxylans (AX) and cellulose were about the same in the two cereals. More marked differences were found for the two bran fractions. Cell wall materials (CWM) of wheat bran comprise pericarp/testa, aleurone and some minor amounts of endosperm tissues (Bacic & Stone, 1981a,b), while CWM of oat bran consist of aleurone, subaleurone and various parts of endospem tissues (Wood, 1986). These differences are clearly reflected in the DF composition; absolute content of lignin and cellulose being much higher in the bran from wheat (86 g/kg and 98 g/kg)

than of oat (30 g/kg and 8 g/kg). The aleurone and subaleurone CWM of oat bran are heavily packed with ß-glucans (Wood, 1986) which is responsible for the high ß-glucan content in this fraction. The composition of rolled oats is between oat bran and flour fractions.

The composition of NSP, in particular the content of ß-glucans, has great impact on the physical properties especially the solubility of NSP. In wheat bran only 15% of the NSP was soluble while in oat bran 59% was soluble (Table 1).

Table 1. Chemical composition of the applied wheat- and oat fractions (g/kg dry matter)

	Flour		Bran		Rolled
	Wheat	Oat	Wheat	Oat	Oats
Starch	829	712	168	424	646
NSP:					
Cellulose	5	4	98	8	14
ß-glucans	4 (4)	22 (19)	14 (12)	98 (77)	42 (38)
AX	22 (11)	21 (8)	287 (46)	43 (11)	29 (8)
Arabinose	8 (4)	7 (2)	94 (11)	15 (4)	11 (4)
Xylose	13 (6)	7 (1)	168 (26)	21 (4)	13 (2)
U.A.	1 (<1)	7 (5)	25 (9)	7 (3)	5 (2)
Total NSP	34 (17)	48 (27)	414 (63)	155 (91)	91 (47)
Klason lignin	1	7	86	30	15
DF	35	56	500	185	106

NSP = non-starch polysaccharides; AX = arabinoxylans; DF = dietary fibre; Values in parentheses are soluble NSP.

Table 2. Digestibility of polysaccharides in the small intestine of pigs fed wheat- and oat based diets

	DF intake	Digestibility, %		
Diet	(g/d)	Starch	NSP	ß-glucans
Expr. 1				
Wheat flour	54	98.7	3	88
Wheat flour + wheat bran	95	98.7	10	64
Wheat flour + oat bran	95	98.6	36	75
Rolled oats + oat bran	173	97.0	34	64
Expr. 2				
Oat flour	90	98.6	27	30
Oat bran	235	98.6	18	18

NSP = non-starch polysaccharides; DF = dietary fibre.

The results from the present pig experiment are consistent with the current physiological knowledge concerning digestion of cereal polysacchardies in various segments of the digestive tract in pigs (e.g Graham et al. 1986a; Bach Knudsen & Hansen, 1990). In spite of the lower starch digestibility of diet ROOB it can be concluded that starch was almost completely digested at the end of the small intestine (Table 2). It can also be concluded that neither insoluble DF (wheat bran) nor soluble DF (oat bran) affect digestibility of starch in the small intestine of pigs.

Thus oat bran, in spite of the fact that it affect the rate of nutrient absorption from the small intestine of both pig and man (Wood, 1990; Bach Knudsen et al. 1990b), did not result in malabsorption of starch in the small intestine. The recovery of wheat NSP at the end of the small intestine was almost complete (90-97%), while there was a significant loss of oat NSP mainly in form of soluble ß-glucans. Similar results were obtained in other studies with pigs fed insoluble and soluble DF sources (e.g. Graham et al. 1986a). When feeding a diet based on cereal grains to pigs, 80% of NSP was recovered in ileal digesta while NSP recovery was raised to 89 % when one third of the basal diet was substitutet with insoluble DF in form of wheat bran. When substituting one third of the basal diet with soluble DF in form of sugar beet pulp, NSP recovery droped to 63 % (Graham et al. 1986a).

In agreement with studies with barley given to pigs (Graham et al. 1986b) there was a significant loss of ß-glucans in the small intestine. In Expr. 1, 64-88 % of oat ß-glucans were digested anterior to the terminal ileum and in Expr. 2 18-30 % (Table 2). However, the fate of oat ß-glucans is different to that of barley. As seen of Figure 1, the ß-glucans of oats are mainly broken down in the ileum segment of the small intestine, where microbial activity reach considerable levels (Bach Knudsen et al. 1990a). This is strongly in contrast to what is found in barley where 26% was broken down at the duodenum presumably by endogenous ß-glucanases (Graham et al. 1986b).

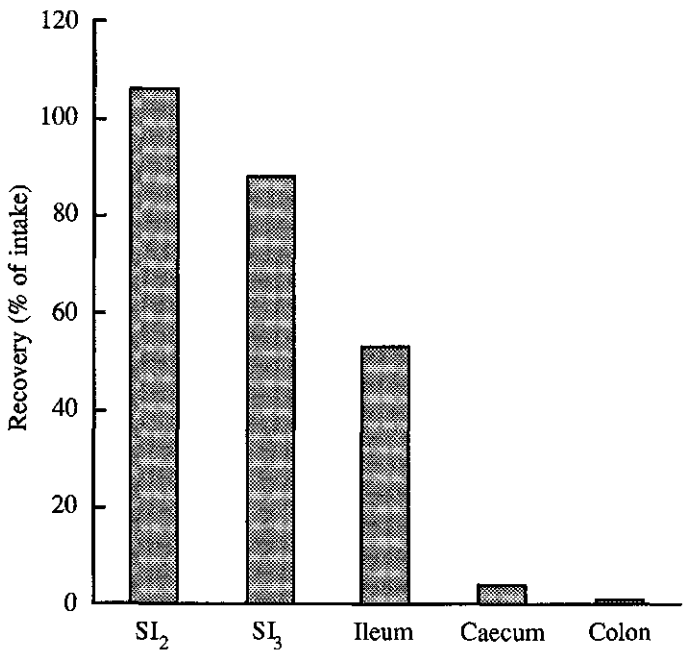

Figure 1. Recovery of ß-glucans at the various sites of the gastrointestinal tract of pigs. The recovery values are average values for the diets: wheat flour + oat bran, rolled oats + oat bran, oat flour and oat bran. SI_2 = midle third of the small intestine, SI_3 = last third of the small intestine.

The general order of breakdown of the various NSP polysaccharides over the entire GI tract was ß-glucans>>AX>cellulose (Table 3). That ß-glucans are a readily fermentable energy source for the microflora in the large intestine is verified by the

data in Figure 1, which shows that 96-99 % of dietary ß-glucans were broken down in caecum and proximal colon and only trace levels survived breakdown over the entire GI tract (Table 3). In contrast to ß-glucans, the breakdown of AX and cellulose is mainly regulated by the physical and chemical structure of CWM explicitly pointed out by the much lower digestibility of AX and cellulose in wheat bran than in oat bran and other nonlignified CWM (wheat and oat flour). As discussed by Bach Knudsen & Hansen (1990), the degree of lignification not only explain the overall digestibility of NSP in the GI tract, but also the relative degradation of AX and cellulose within the various type of CWM.

Table 3. Digestibility of non-starch polysaccharides in the digestive tract of pigs fed wheat- and oat based diets

Diet	DF intake (g/d)	Digestibility, % NSP	Cellulose	ß-glucans	AX
Expr. 1					
Wheat flour	54	85	38	100	87
Wheat flour + wheat bran	95	62	44	100	62
Wheat flour + oat bran	95	91	83	100	90
Rolled oats + oat bran	173	92	83	100	84
Expr. 2					
Oat flour	90	87	65	100	83
Oat bran	235	88	71	100	83

NSP = non-starch polysaccharides; AX = arabinoxylans; DF = dietary fibre.

The digestibility of polysaccharides and other major constituents are important factors to consider when discussing the GI implications of various DF sources. In keeping with other studies (Stephan & Cummings, 1980), lignified DF sources (wheat bran) are only degraded to a small extent resulting in an increase in faecal dey matter and bulk by virtue of its physical presence and its water holding capacity. In colon these DF sources caused a dilution of the content. In contrast oat DF was extensively degraded in the large intestine. This stimulation of microbial activity (Bach Knudsen & Jensen, 1991) lead to an increased microbial biomass in feacal materials and a more soft faeces. The same was found with cell walls from other soluble DF sources: cabbage, pectins, and carrots (Stephen & Cummings, 1980).

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DIGESTION OF PLANT CELL WALL POLYSACCHARIDES IN PIGS FED ON DIETS VARYING IN CELL WALL AND LACTOSE CONTENTS

Elisabeth CHABEAUTI*, Yolande JAGUELIN*, C.FEVRIER*, B.CARRE** and Y. LEBRETON*

* I.N.R.A.-S.R.P., Saint-Gilles 35590 L'Hermitage
** I.N.R.A.-S.R.A., Nouzilly 37380 Monnaie

Abstract

Ileal and fecal apparent digestibilities (AD) of plant cell walls were measured on Large White castrated growing pigs fed on diets containing wheat for the control diets (about 7% of WICW) and wheat bran or sugar beet pulp for the fibre diets (about 15% of WICW), either alone (diets WS, BS and PS) or in combination with lactose (diets WL, BL and PL).

Fibre from sugar beet pulp was better degraded than those from wheat bran at the end of the ileum as well as in the overall tract. The addition of lactose significantly depressed the ileal and fecal AD of GE and N. But the ileal AD of dietary fibre in all the diets and the fecal AD of wheat bran and sugar beet pulp plant cell walls were not affected.

Keywords: pig, digestibility, lactose, dietary fibre, non-starch polysaccharides.

Introduction

The nutritive value of the pig diets is more and more precisely known, but the effects of the interactions between some unconventional feedstuffs are still a challenge for an accurate computerization of diets. These problems are more frequent with the increasing use of agro-industrial by-products. The more widely studied interactions are those relative to the presence of dietary fibre on the apparent digestibility (AD) of the nutrients in growing pigs (Drochner, 1984; Laplace et al, 1989). But highly fermentative components, such as whey, can also have such interactive effects (Cheeke & Stangel, 1973; Jost et al, 1982).

Therefore, the present work was designed to study the ileal and fecal AD of non-starch polysaccharides from two fibre sources, wheat bran or sugar beet pulp, either alone or in combination with lactose. The classical determinations of the plant cell walls as Van Soest's splitting up were precised by the more exact knowledge of their non-starch-polysaccharides (Carré et al, 1990).

Materials and Methods

Diets

A basic diet WS was mainly made with wheat (60%) and starch (25%), cc plemented with animal protein (5% of soluble fish protein concentrate and 5% of hydrochloric casein with 5% minerals and vitamins). From this diet, wheat bran (28.5%) or sugar beet-pulp (19.7%) partially replaced wheat to formulated two diets BS and PS respectively, in order to contain 15 p.cent of WICW (Water Insoluble Cell Wall). Lactose (25%) replaced starch in three other diets WL, BL and PL.

Fecal digestibility

24 castrated male growing pigs weighing on average 59.4 kg at 116 days of age were allocated to the diets, according to a complete block design. After a 14-day period of adaptation to experimental conditions, the total fecal collections were performed during the following 10 days (Chabeauti et al, 1991). The animals were pair-fed twice a day with wet diets (2 water/1 feed) supplying a similar amount of calculated digestible energy for starch diets (28.5 MJ/d).

Ileal digestibility

A termino-terminal ileorectal anastomosis was performed on 4 castrated male growing pigs, for ileal digestibility measurements (Laplace et al., 1989). After a 3-week post-operative period of recovery, the pigs were housed in the same conditions as for fecal collection. Each pig was allocated to each diet during two weeks and the ileal effluents were quantitatively collected during the second week. Their mean initial weight was 34.4 kg for 147 days of age.

Chemical analysis

Ash, nitrogen and gross energy were determined in feeds and digesta according to AOAC methods (1975). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were evaluated in feeds and digesta according to the modified Van Soest's fibre splitting up for starch-rich feeds (Giger & Pochet,1987). The WICW (Carré & Brillouet, 1989) was applied on feeds. The non-starch polysaccharides (NSP) were determined in feeds and digesta by gas-liquid chromatography of the alditol acetates from polysaccharide monomers as described by Carré et al. (1990). Uronic acids were determined from acid hydrolysates of samples using the m-phenylphenol method, with galacturonic acid as a standard (Blumenkrantz & Asboe-Hansen,1973).

Calculation and statistical analysis

The General Linear Model of the Statistical Analysis System (1987) including an analysis of variance, the Duncan's test and the method of contrasts to test the interactive effects was used for the statistical analysis.

Results

Composition of plant cell walls in diets (table 1)

In all the diets, NDF amounts were lower than the WICW ones, the largest difference occurring with the pulp diets (38,0 g/kg vs 15,5 g/kg for wheat bran diets). Total-NSP contents of diets were expressed as the sum of NSP from water soluble cell walls and from WICW. The average ratio of soluble NSP to total-NSP varied according to the different plant cell walls used in the diets: 0.067 for wheat bran, 0.117 for wheat and 0.174 for sugar beet pulp. The diets containing wheat or wheat bran had a similar total-NSP composition with 43 % or 45 % for xylose and 4% or 5% for uronic acids, respectively. They differed significantly from sugar beet pulp diets whose total-NSP contained only 13 % of xylose but 19 % of uronic acids.

TABLE 1: CHEMICAL ANALYSIS OF THE EXPERIMENTAL DIETS g/kg dry matter

DIETS	WS	WL	BS	BL	PS	PL
NDF	57	58	136	133	126	116
ADF	25	23	50	48	67	64
ADL	7	7	13	13	10	9
WICW	71	66	150	150	158	161
Arabinose	13	12	27	27	37	37
Xylose	25	23	55	54	19	19
Galactose	3	3	4	5	9	12
Glucose	14	13	29	29	45	44
Uronic Ac.	3	2	6	6	27	27
Total-NSP	58	54	120	121	143	142

Apparent digestibility of main nutrients (table 2)

Increasing plant cell wall amount significantly lowered the fecal and ileal AD of energy and nitrogen. This was reinforced by the presence of lactose in place of starch (except for ileal AD values from pulp diets). Moreover, the fecal AD of energy and nitrogen was significantly lower in the BS and BL diets than in the PS and PL ones, but this difference wasn't noticed at the ileum. However, 35% and 41% of ingested lactose reached the end of the ileum with WL and BL diets but only 18% with PL diet.

TABLE 2: APPARENT DIGESTIBILITY OF MAIN NUTRIENTS (1)

DIETS		WS	WL	BS	BL	PS	PL	A	S	L	A*L	S*L	SEM
GE	I	86a	75b	74b	60c	74b	68b	**	**	**	NS	NS	4
	F	**92a**	**91a**	**84d**	**82e**	**88b**	**87c**	**	**	**	**NS**	**NS**	**1**
N	I	85a	81ab	80ab	72c	76bc	71c	**	NS	**	NS	NS	4
	F	**92a**	**88b**	**87bc**	**79d**	**84c**	**78d**	**	**NS**	**	**NS**	**NS**	**2**

(1)- NS: non significant; *: P<0.05; **: P<0.01; data with the same superscript on the same ligne are not significantly different between diets.
(2)- A: effect of plant cell wall amount; S: effect of plant cell wall source; L: effect of lactose addition; A*L and S*L: interaction effects; S.E.M.:standard error of the mean.

Apparent digestibility of plant cell walls (table 3)

By using the Van Soest's fibre splitting up, the ileal AD of hemicellulose (NDF-ADF) was significantly decreased by lactose only for the pulp diets. Its fecal AD was higher in PS and PL diets than in BS and BL ones. In an other way, the ileal AD of cellulose (ADF-ADL) was higher for wheat bran diets than for pulp ones. Interactions with lactose

occurred on its fecal AD, with the level of fibre as well as with its origin.

TABLE 3: APPARENT DIGESTIBILITY OF PLANT CELL WALLS (1)

DIETS		WS	WL	BS	BL	PS	PL	A	S	L	A*L	S*L	SEM
NDF-ADF	I	-1b	-3b	-1b	1b	39a	-13b	NS	NS	*	NS	**	13
	F	**41c**	**47b**	**45b**	**45b**	**71a**	**70a**	**NS**	**	*	**	**NS**	**2**
ADF-ADL	I	29a	23a	22a	16ab	4b	5b	NS	**	NS	NS	NS	9
	F	**45b**	**45b**	**30c**	**24d**	**76a**	**81a**	**	**	**NS**	*	**	**3**
Arabin.	I	28abc	19bc	-5d	17c	37ab	43a	**	**	NS	*	NS	12
	F	**55b**	**51b**	**42c**	**43c**	**88a**	**87a**	**	**	**NS**	**NS**	**NS**	**5**
Xylose	I	24ab	13bc	3c	20bc	25ab	38a	NS	**	NS	*	NS	11
	F	**72**	**69**	**67**	**66**	**69**	**67**	**NS**	**NS**	**NS**	**NS**	**NS**	**4**
Glucose	I	39c	35c	44bc	36c	62a	60ab	NS	**	NS	NS	NS	11
	F	**65b**	**56bc**	**62bc**	**52c**	**84a**	**90a**	**NS**	**	**NS**	**NS**	*	**7**
Uronics	I	-15	3	-15	3	-17	-23	NS	NS	NS	NS	NS	21
	F	**42b**	**25c**	**29c**	**31c**	**93a**	**92a**	**NS**	**	*	**	**NS**	**5**
T-NSP	I	28	19	9	19	32	34	NS	NS	NS	NS	NS	12
	F	**65b**	**59c**	**58c**	**55c**	**85a**	**85a**	**	**	*	**NS**	**NS**	**3**

(1)- NS: non significant; *: P<0.05; **: P<0.01; data with same supercript on the same ligne are not significantly different between diets.
(2)- A: effect of plant cell wall amount; S: effect of cell wall source; L: effect of lactose addition; A*L and S*L: interaction effects; SEM: standard error of the mean.

The ileal AD of arabinose and xylose from bran diets was improved by lactose while it was depreciated for wheat ones. Total-NSP and individual oses from pulp diets were more digestible than those from wheat bran diets at the end of the ileum (except for their uronic acids which were not digested) as well as for the overall digestive tract (except for xylose). Lactose decreased the fecal AD of total-NSP only for wheat diets but did not affect the utilization of neutral oses and total-NSP from other diets.

Discussion

The important decrease of ileal and fecal AD of major nutrients by an increase of levels of dietary fibre had to be modulated with their botanical origin, in good agreement with classical results (Stanogias & Pearce, 1985; Graham et al, 1986). Nevertheless, the lack of variation of ileal and fecal AD of N between wheat bran and sugar beet pulp diets is more controversial (Graham et al, 1986; Low et al, 1988).

Our results concerning the grading of the ileal and fecal AD values for wheat, wheat bran and sugar beet pulp based diets were also reported by Graham et al (1986), Low et al (1988) and Laplace et al (1989). The observed differences can be related to the chemical composition (high digestible pectic substances in the pulp, lesser digestibility of

hemicelluloses in wheat and wheat bran, high lignin content in bran) and to the physical form of plant cell walls (Chabeauti et al, 1991).

Numerous authors observed degradation of fibre at the end of the ileum in growing pigs, varying with the plant cell wall origin and components (Drochner, 1984; Graham et al, 1986; Laplace et al, 1989). Thus, for a same amount of WICW, considering the ratio of ileal AD to fecal AD of total-NSP or of NDF, the wheat bran diets were better fermented in the large intestine than the beet pulp diets as already pointed out by Graham et al (1986). This explains that the differences observed in the ileal digestion of xylose between these diets could no longer be observed after the fermentation in the large intestine.

The depressive effect of lactose on the fecal digestibility of major nutrients is in good agreement with other previous results obtained with whey (Cheeke & Stangel, 1973; Jost et al, 1982; Février & Lachance, 1988). But, the interactive effect is less important, probably because whey contains also high levels of minerals which increase the osmotic pressure, reduce the intestinal transit time and further depress the digestive process (Low, 1988).

The fecal AD of total-NSP and individual oses from wheat based diets decreased with the adding of lactose. Thus, it would seem that the priority of degradation by the intestinal bacteria of the hindgut was given to the lactose instead of wheat fibres (Shearer et al, 1969; Thévenet, 1988). But, this effect was not sufficient enough to influence fecal digestibility of plant cell walls from high fibrous diets. This could be explained, firstly, by the decrease of the transit time associated with high levels of fibre (Furuya & Takahashi, 1975) allowing an increased capacity of fermentation of plant cell walls (wheat bran and pulp) and, secondly, by the high degree of digestibility of some plant cell walls (pulp). Moreover, lactose did not seem to interfer with plant cell wall breakdown up to the caecum but the higher the ileal digestion of fibre was, the higher the degradation of lactose.

From these digestibility data, including sugar beet pulp in a diet containing lactose seem to be more suitable than adding wheat bran for dietary fibre supply.

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EFFECT OF VARIOUS SOURCES OF DIETARY FIBRE ON CHEMICO-PHYSICAL CHARACTERISTICS OF DIGESTA IN THE STOMACH AND THE SMALL INTESTINE OF THE PIG

J. van der Meulen & J.G.M. Bakker

Research Institute for Livestock Feeding and Nutrition (IVVO), Lelystad, The Netherlands

Abstract

The effect of including dietary fibre from various sources in a basal low-fibre diet on the amount of digesta, pH, osmolarity, redox potential and volatile fatty acid (VFA) concentration in the stomach and the small intestine was studied. Eighteen finishing pigs (78 kg) were fed for 3 weeks a basal low-fibre (80 g NDF/kg) diet without or with 20% wheat bran, sunflower meal, beet pulp, soybean hulls or pea hulls. Immediately after killing (4.5 h after feeding), samples of digesta were taken from the stomach and 4 sections of the small intestine. The amount of digesta in the stomach and the small intestine increased by including fibre, particularly wheat bran, sunflower meal and pea hulls. The pH in the stomach and the 2 proximal sections of the small intestine decreased by inclusion of dietary fibre. The redox potential decreased along the small intestine. Compared with the stomach the osmolarity in the small intestine was higher. The VFA concentration in the stomach was low and increased along the small intestine, but was not affected by inclusion of fibre in the diet.

Introduction

Nowadays there is still considerable interest in the use of high-fibre by-products of the food industry in feeds for pigs. The structural polysaccharides of these high-fibre by-products are resistant to the mammalian enzymes, but are fermented to a greater or lesser extent by the microflora in the gastrointestinal tract. This fermentation occurs largely in the caecum and the colon (Argenzio & Southworth, 1975). Before reaching the large intestine the polysaccharides have to pass the stomach and the small intestine, and may there affect the chemico-physical conditions. Changes in the chemico-physical conditions in the stomach and the small intestine in turn may affect the efficiency of enzymes, which are supplemented to improve the utilization of pig diets (Chesson, 1987).
In this study the effect of including dietary fibre from various sources on pH, osmolarity, redox potential, conductivity and volatile fatty acid (VFA) concentration in the stomach and the small intestine of finishing pigs was investigated.

Materials and Methods

Eighteen finishing Dutch Landrace pigs with an initial weight of 77.7±4.6 kg were used in 3 periods. In each period 6 pigs were housed individually and fed twice daily (07:00 and 16:00 h) a basal low-fibre diet without (B) or with 20% wheat bran (Wb), sunflower meal (Sm), beet pulp (Bp), soybean hulls (Sh) or pea hulls (Ph)(Table 1). After 3 weeks the animals were killed 4.5 hours after last feeding. Immediately after

killing the small intestine was separated by ligatures into 4 equal sections (numbered 1 to 4 from proximal to distal end), and the stomach and the small intestine were removed. The contents of stomach and each section of the small intestine were collected and weighed. Immediately after collection of the digesta, the pH and the redox potential were determined. Thereafter, the samples were divided in two subsamples and stored at -20°C. In the first subsample dry matter content was determined. The second subsample was centrifuged (1000 *g*, 10 min) to measure the amount of free water. In the obtained supernatant conductivity, osmolarity and VFA concentration were determined.

Table 1. Composition and chemical analysis of the 6 diets (B=basal, Wb=wheat bran, Sm=sunflower meal, Bp=beet pulp, Sh=soybean hulls, Ph=pea hulls).

	Diet					
Ingredients (g/kg)	B	Wb	Sm	Bp	Sh	Ph
Maize starch	200					
Wheat bran		200				
Sunflower meal			200			
Beet pulp				200		
Soybean hulls					200	
Pea hulls						200
Maize	320	320	320	320	320	320
Tapioca meal	200	200	200	200	200	200
Herring meal	50	50	50	50	50	50
Meat meal	50	50	50	50	50	50
Soybean	140	140	140	140	140	140
Soybean oil	18	18	18	18	18	18
DL-Methionine	2	2	2	2	2	2
Vitamin-mineral premix	20	20	20	20	20	20
Chemical analysis						
Crude protein	242	265	254	292	301	259
NDF	79	179	170	154	159	183
NDADF	14	48	59	67	74	110

Results and Discussion

The proximal section of the small intestine contained little digesta compared with the other sections. This is in agreement with results of Clemens et al. (1975), who recovered less fluid and digesta markers from the proximal than from the distal part of the small intestine. Amounts of fresh and dried digesta from the stomach and the small intestine of the pigs fed the basal diet were less than in the pigs fed the high-fibre diets, however this was only significant for the content of the stomach (Table 2). When they increased the NDF level of the feed up to 300 g/kg, Stanogias & Pearce (1985) observed a significant increase in the amounts of dried digesta from both the stomach and the small intestine.
In the stomach the pH ranged from 3.2-4.5 and increased along the small intestine, reaching values of 6.4-6.8 in the distal section (Table 3).

Table 2. The mean (n=3) amounts of fresh and dried digesta from the stomach and the small intestine.

	Diet					
	B	Wb	Sm	Bp	Sh	Ph
Amount of fresh digesta (g)						
stomach	1150^{a}	1925^{c}	1360^{b}	1525^{b}	2422^{c}	1900^{c}
small intestine	631	982	927	728	1039	839
Amount of dry digesta (g)						
stomach	217^{a}	465^{c}	271^{ab}	306^{ab}	553^{c}	397^{bc}
small intestine	85	106	109	106	122	132

Values with a different superscript differ significantly ($P<0.05$).

Table 3. The mean (n=3) pH and redox potential in the stomach and the small intestine.

	Diet					
	B	Wb	Sm	Bp	Sh	Ph
pH						
stomach	4.51^{a}	3.83	3.94	3.49^{b}	3.61^{b}	3.26^{b}
small intestine 1	6.29	5.86	5.86	6.06	6.04	6.04
small intestine 2	6.43	6.32	5.95	6.39	6.09	6.15
small intestine 3	6.50	6.28	6.40	6.55	6.66	6.42
small intestine 4	6.58	6.68	6.80	6.56	6.59	6.43
Redox potential (mV)						
stomach	- 37	-165	- 47	-176	-151	- 48
small intestine 1	-166	-397	- 64	-394	-348	-259
small intestine 2	-345	-410	-167	-449	-396	-337
small intestine 3	-419	-532	-284	-512	-485	-426
small intestine 4	-404	-493	-513	-490	-479	-388

Values with a different superscript differ significantly ($P<0.05$).

This agrees with the pH-values measured by Argenzio & Southworth (1975) and Clemens et al. (1975). The pH of the gastric digesta from the pigs fed the high-fibre diets was lower than in the pigs fed the basal diet, but this was only significant when beet pulp, soybean hulls or pea hulls were fed. Also, in the 2 proximal sections of the small intestine the pH was lower in the pigs fed the high-fibre diets, but this was not significant (Table 3). After feeding a low-protein, high-cellulose diet Argenzio & Southworth (1975) observed lower pH-values in the stomach and the proximal small intestine. Increasing the fibre level of maize-based diets by adding coarse bran also lowered the pH level in the stomach (Lawrence, 1972), although Kass et al. (1980) observed no unequivocal effect of increasing the level of alfalfa meal on the pH level in the stomach and the small intestine. In experiments aimed to improve the

Table 4. The mean (n=3) percentage of free water, osmolarity, conductivity and VFA content in the stomach and the small intestine.

	Diet					
	B	Wb	Sm	Bp	Sh	Ph
Free water (% of wet weight)						
stomach	54.3	44.1	32.8	44.2	48.6	51.2
small intestine 1	59.0	65.3	84.6	78.7	79.4	64.9
small intestine 2	63.9	65.9	44.4	57.8	65.1	49.2
small intestine 3	49.1	51.2	36.2	56.4	52.2	48.3
small intestine 4	54.3^{a}	52.4^{a}	24.5^{b}	39.7	48.0^{a}	38.8
Osmolarity (Osm)						
stomach	0.34	0.39	0.44	0.31	0.35	0.32
small intestine 1	0.58	0.54	0.51	0.54	0.53	0.55
small intestine 2	0.58	0.48	0.54	0.62	0.52	0.58
small intestine 3	0.54	0.51	0.49	0.50	0.46	0.52
small intestine 4	0.51	0.50	0.41	0.45	0.43	0.43
Conductivity (mS/cm)						
stomach	10.85	10.97	10.49	10.63	11.09	10.52
small intestine 1	8.40	9.44	10.23	9.07	10.23	9.69
small intestine 2	8.45^{a}	9.09	9.72^{b}	9.49^{b}	10.30^{b}	9.43
small intestine 3	9.04^{a}	8.96^{a}	9.77	8.96^{a}	10.14^{b}	9.43
small intestine 4	8.64	9.38	9.85	9.24	9.91	9.80
VFA (mmol/l)						
stomach	12.3^{b}	9.6^{b}	25.5^{a}	13.6^{b}	13.4^{b}	8.7^{b}
small intestine 1	3.9	22.1	6.5	6.5	10.4	7.7
small intestine 2	14.3	16.1	5.7	15.1	14.2	11.4
small intestine 3	26.0^{b}	26.9^{b}	10.6^{a}	31.0^{b}	34.9^{b}	37.1^{b}
small intestine 4	61.7	56.4	27.6	54.7	44.5	45.9

Values with a different superscript differ significantly ($P<0.05$).

availability of nutrients from fibrous by-products by enzyme supplementation, the observed pH decrease in the stomach and the proximal small intestine has to be taken into account to attain optimum enzyme activity.

The redox potential in the stomach varied from -30 to -180 mV and decreased along the small intestine. The redox potential in the stomach and the small intestine did not differ between pigs fed the basal and the high-fibre diets (Table 3). The decrease in the redox potential along the small intestine may be caused by absorption of reducing substances (Sambrook, 1980) and an increase in bacterial fermentation resulting in an increase in reduced substances.

The percentage of free water was higher in the proximal part of the small intestine (59-84%) than in the stomach (32-54%), and decreased along the small intestine till 24-54% in the distal section. The osmolarity pursued almost the same course along the stomach and the small intestine as the percentage of free water. The conductivity was high in the stomach (10.5-11.0 mS/cm) and remained relatively constant (8.4-10.3 mS/cm) in the small intestine. No significant difference was observed in the

percentage of free water, osmolarity and conductivity in the stomach and the small intestine between pigs fed the basal and the high-fibre diets (Table 4). However, in all the pigs fed the high-fibre diets the percentage of free water in the proximal section of the small intestine was higher than in the pigs fed the basal diet. This may be caused by the endogenous secretion, which increases by feeding high-fibre diets (Partridge et al., 1982; Langlois et al., 1987; Zebrowska & Low, 1987). An increased endogenous secretion may also cause a higher conductivity in the small intestine of the pigs fed the high-fibre diets. The low percentage of free water in the distal sections of the small intestine in the pigs fed the high-fibre diets containing beet pulp, may be caused by the high water-binding capacity of pectins in beet pulp.
The VFA concentration was low in the stomach and in the proximal part of the small intestine. The VFA concentration increased along the small intestine till 28-61 mmol/l at the distal end. No difference was found between pigs fed the basal and the high-fibre diets (Table 4). The increase in VFA concentration along the small intestine indicates an increase in bacterial fermentation. Although Argenzio & Southworth (1975) and Clemens et al. (1975) did not observe such an increase at the end of the small intestine, measurement of the ATP concentration also indicates an increase in microbial activity in the distal part of the small intestine (Jensen, 1988). Both starch from the basal diet and non starch polysaccharides from the fibrous diets may be used as energy sources for this microbial fermentation.

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ILEAL AND FAECAL DIGESTIBILITY OF POLYSACCHARIDES IN PIGS FED DIETS WITH DIFFERENT VARIETIES OF PEA

Jolanta Gdala[1], H. Graham[2], Lucyna Buraczewska[1] and P. Åman[2]
[1]Institute of Animal Physiology and Nutrition, Jabłonna, Poland
[2]Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Uppsala, Sweden

Abstract

Nine varieties of white- and coloured-flowered pea were analysed for content of protein, starch, dietary fibre and fiber fractions, and used in trials on cannulated growing pigs to evaluate ileal and faecal digestibility of carbohydrates. Pigs were prepared with a T-piece cannula inserted in the terminal ileum. Each diet contained 51.7% of barley and 41.5% of pea. Starch was the most variable component in the peas (30-42%). Coloured-flowered peas contained more NDF and ADF but not dietary fiber and especially more lignin than the white-flowered peas. Ileal digestibility of dietary starch and dietary fiber ranged from 85 to 93 and from 13 to 37%, respectively. Total degradation of dietary fiber and its fractions was higher in the white- than in the coloured--flowered peas. For all the barley-pea diets, digestibility coefficient of total dietary fiber ranged from 47 to 87.
Keywords : ileal digestibility, pigs, polysaccharides, pea.

Introduction

Pea provides an important source of highly digestible protein and starch in diets for pigs. However, about one quarter of pea dry matter consists of non-starch polysaccharides, oligosaccharides and lignin which are not digested by the digestive enzymes of the pig. White- and coloured-flowering varieties of pea differ in their tannin content and ileal digestibility of protein (Buraczewska et al., 1989). There is not much evidence available on the ability of pig microflora to ferment pea fibre-components along the digestive tract. The aim of the study was to compare the apparent digestibility of polysaccharides of two types of pea (nine varieties), measured at the terminal ileum and over the entire digestive tract of pigs.

Materials and Methods

Large White x Landrace castrated male pigs of 30-80 kg with T-shape ileal cannula were offered ten barley-pea diets with six white-flowered varieties of pea (Belinda, batches 1 and 2, Kaliski, Mige, Opal, Milewska and Koral) and three varieties with coloured frowers (Matmal, Mazurska and Gomik). All the peas were spring varieties and had smooth seeds. Each diet containing 41.5% of the tested pea, supplemented with minerals

and vitamins, was offered every 12 h to 4-6 pigs for 2 weeks at 3.8% body weight/day. Chromium oxide was used as an indicator. After seven days of preliminary period, faeces (3 days) and digesta (at least 3 x 12 h) were collected. The peas, diets and freeze-dried collected samples from each animal were analysed for starch, NDF and ADF content. However, for analysis of dietary fibre and Klason lignin only pooled samples of digesta and faeces from pigs fed on the same diet were prepared. NDF and ADF were determined using the Tecator Fibertec System M., and the other components according to Theander and Westerlund procedure (1986).

Results and Discussion

Chemical analysis of the peas (Tables 1 and 2) showed that there were no great differences between the two types of pea. The coloured-flowered varieties contained more NDF and especially more Klason lignin. One of the most variable component in all the peas was starch (30 to 42%) which is consistent with observations of Savage et al. (1989). The starch content tended to be inversely correlated with dietary fibre content of the peas.

Table 1. Chemical composition of Polish peas, % of DM (CP - crude protein; EE-ether extract; S- starch; DF - dietary fibre; CF - crude fibre; R - the rest to 100%).

Pea	CP	EE	Ash	S	DF	NDF	ADF	CF	R*
White-flowered									
Belinda 1	27.7	1.81	3.62	na**	22.8	11.8	9.0	6.8	na
Kaliski	26.8	1.90	3.28	na	26.0	12.7	10.6	7.6	na
Mige	26.1	1.43	3.30	42.4	21.6	11.7	7.6	6.1	5.2
Belinda 2	26.1	1.40	3.58	38.4	23.4	13.4	8.1	6.4	7.1
Opal	22.4	1.85	3.25	40.6	25.8	12.6	7.6	5.4	9.8
Milewska	22.6	1.85	3.20	34.8	24.5	15.7	9.6	7.2	13.1
Koral	22.1	1.94	3.13	37.0	21.7	12.6	8.3	5.8	14.1
Coloured-flowered									
Matmal	26.6	1.57	2.69	30.5	26.1	17.2	10.9	7.4	12.5
Mazurska	22.7	2.18	3.30	33.0	23.0	14.3	9.8	6.6	15.8
Gomik	21.1	1.58	3.60	38.7	22.5	15.8	10.2	7.4	12.4

* R=100-(CP+EE+A+S+DF); ** na - not analyzed

NDF accounted for 49 to 70% of dietary fibre while respective value for ADF accounted on average for 39%. Not analysed part of dry matter (R) ranged from about 5 to 16% in the peas. This fraction can involve 5-8% raffinose family oligosaccharides, which causes flatulence, and also some sucrose (Saini, 1988).

The monosaccharide composition of dietary fibre showed dominating content of glucose and relatively about five times less of arabinose and uronic acid. Still lower levels, showed

in decreasing order, were found for galactose, xylose, rhamnose and mannose.

As is shown in Table 3, starch content was higher and dietary fibre lower in the barley-pea diets than in the peas; NDF and ADF were of similar level.

Table 2. Content of dietary fibre monosaccharides and Klason lignin in the tested peas (% of DM).

Pea	Monosaccharides of dietary fibre							
	Glc*	Ara	UroA	Gal	Xyl	Rha	Man	KL
White-flowered								
Belinda 1	14.91	2.33	2.24	1.39	0.72	0.30	0.29	0.62
Kaliski	18.11	2.40	2.12	1.02	0.98	0.28	0.28	0.81
Mige	12.33	2.19	2.68	1.63	0.89	0.36	0.39	1.10
Belinda 2	14.36	2.35	2.80	1.62	0.78	0.32	0.43	0.70
Opal	16.24	2.85	2.77	1.68	0.89	0.41	0.39	0.60
Milewska	14.35	2.95	2.94	0.99	1.25	0.27	0.24	1.50
Koral	12.08	3.65	2.68	0.92	1.35	0.36	<0.20	0.65
Coloured-flowered								
Matmal	16.10	2.05	2.59	1.58	0.83	0.40	0.48	2.10
Mazurska	12.57	2.66	2.78	0.83	0.99	0.26	0.31	2.56
Gomik	12.28	2.76	3.09	1.00	1.14	0.25	0.23	1.78

*Glc - glucose; Ara - arabinose; UroA - uronic acid; Gal - galactose; Xyl - xylose; Rha - rhamnose; Man - mannose; KL - Klason lignin

Table 3. The content of starch, dietary fibre, NDF and ADF in the barley-pea diets (% of DM).

Diets with pea:	Starch	Dietary fibre	NDF	ADF
Belinda 1	51.4	17.7	12.5	7.0
Kaliski	48.5	18.1	13.4	6.6
Mige	49.0	18.3	13.8	6.3
Belinda 2	47.6	18.7	14.3	6.7
Opal	50.7	18.6	13.0	6.2
Milewska	49.0	19.0	14.8	7.1
Koral	47.0	18.5	16.2	6.7
Matmal	46.4	18.5	18.3	7.6
Mazurska	48.1	17.0	13.2	7.0
Gomik	48.5	18.0	14.3	7.2

Ileal and total digestibility of starch, dietary fibre, ADF and Klason lignin is presented in Table 4. From seven to 15% of starch was not digested up to the end of the small intestine. It seems unlikely that the digestibility of the starch

in peas was being limited by the presence of inhibitors of the digestive enzymes. Amylase inhibitors have never been detected in peas (Liener, 1989).

The results show that in growing pigs the non-starch polysaccharides of barley-pea diets are degraded to a considerable degree and clearly, part of it can be fermented in the small intestine. The mean values and the range of total digestibility of dietary fibre were higher for the diets with white-flowered (61-87%) than wth coloured-flowered peas (47-56%). The lower digestibility can be explained by higher content of lignin and tannins (Gdala, 1990) in this type of pea.

In the small intestine glucose and galactose dissapeared to the higher extent then the other monosaccharides of the dietary fibre. Xylose was the less fermentable component of fibre along the whole digestive tract. However, the precision of estimation its digestibility is low because only small amounts of this saccharide was detectable.

Table 4. Ileal and total digestibility of starch, fibre components and Klason lignin in pigs fed barley-pea diets.

Diets with:	White flowered pea /n=7/				Coloured flowered peas /n=3/			
Digestibility:	ileal		total		ileal		total	
	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range	$\bar{x}$	range
Starch	88	85-93	100		87	85-91	100	
Dietary fibre	32	20-37	69	61-87	20	13-24	51	47-56
Monosaccharides of dietary fibre:								
arabinose	22	0-31	78	71-84	15	0-25	78	75-82
xylose	17	0-27	45	31-60	12	-14-19	40	34-47
galactose	33	16-75	74	48-83	16	9-29	71	66-77
glucose	47	35-55	71	58-77	41	38-44	58	54-61
uronic acid	25	21-29	80	75-85	10	0-16	65	56-72
ADF	20	9-29	49	41-64	14	-7-29	28	18-43
Klason lignin	4	0-25	27	0-49	9	0-24	10	0-24

Table 5 shows that several diets differed significantly in NDF digestibility. Degradation of NDF was up to 48% in the small intestine and up to 67% in the whole digestive tract. The variation in results may be due to uncertainty in the analysis. Generally, the total degradability of NDF tended to be lower than that of dietary fibre.

It is generally believed that in dicotyledons such as peas, polysaccharides in the primary cell walls like pectin and xyloglucan are much more readily degradable than those prevailing in the secondary walls like cellulose and acidic xylan (Graham et al., 1985; Nordkvist and Åman, 1986). Pea xylan was found to be largely undigested also by cockerels (Longstaff and McNab, 1987).

No statistical differences were found in NDF digestibility for pigs within a body weight from 30 to 70 kg (Table 6),

Table 5. Ileal and total digestibility of NDF in the barley-pea diets fed to pigs.

Diet with pea	Digestibility, %	
	Ileal	Total
Belinda 1	3.9^{A}	45.7^{AB}
Kaliski	35.4^{BCD}	58.0^{CDE}
Mige	33.5^{BC}	57.2^{CD}
Belinda 2	31.6^{BC}	56.4^{CD}
Opal	19.5^{B}	55.6^{CD}
Milewska	35.9^{CD}	66.6^{E}
Koral	48.0^{D}	63.1^{DE}
Matmal	29.1^{BC}	52.0^{BC}
Mazurska	22.7^{BC}	37.7^{A}
Gomik	29.2^{BC}	50.1^{BC}

Values in columns with different letters are significantly different ($P < 0.01$)

Table 6. Mean ileal and total digestibility of NDF in all the experimental diets fed to pigs with different range of body weight.

Weight of pigs, kg	n	Daily intake	Digestibility			
			Ileal		Total	
			g/day	%*	g/day	%*
30-40	5	160.4	42.8	26.7	73.0	45.5
40-50	14	229.5	87.9	38.3	126.0	54.9
50-60	18	254.4	96.7	38.0	147.0	57.8
60-70	13	304.6	108.0	35.5	184.0	60.4

* Regression analysis showed that differences were not significant

and fed the barley-pea diets containing both the white- and the coloured-flowered types of pea.

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EFFECT OF SUPPLEMENTATION OF PROPIONIC ACID ON ILEAL AND FECAL NUTRIENT AND ENERGY DIGESTIBILITIES AND ON THE LEVEL OF MICROBIAL METABOLITES IN ILEAL AND CECAL DIGESTA OF GROWING PIGS

R. Mosenthin[1], W. C. Sauer[2], F. Ahrens[3] and C. F. M. de Lange[2]

1. Institute of Animal Nutrition and Feed Science, University of Kiel, D-2300 Kiel, Germany
2. Department of Animal Science, University of Alberta, Alberta, T6G 2P5, Canada
3. I. S. Research Institute for Animal Physiology and Nutrition D-2362 Wahlstedt, Germany

Abstract

The supplementation of propionic acid to diets for growing pigs tends to increase both the ileal and fecal digestibilities of crude protein and energy as well as the ileal digestiblilities of most of the amino acids. The addition of propionic acid to a basal diet decreased ($p<.05$) the level of ammonia in ileal digesta from 940.9 to 720.4 µg/g DM and caused a trend towards a lower level of cadaverine and putrescine. In addition, there was a decrease ($p<.05$) in the level of cadaverine in cecal digesta from 821.0 to 116 nmol/g DM as well as a trend towards a lower level of ammonia and putrescine. The effect of propionic acid supplementation on the parameters measured was less pronounced when added in combination with siliceous earth.
Keywords: Propionic acid, pigs, digestibility, amines, ammonia, amino acids.

1. Introduction

There is growing evidence that the supplementation of some organic acids may improve both the performance of starter (Kirchgessner and Roth, 1976; Falkowski and Aherne, 1984; Giesting and Easter, 1985) and grower-finisher pigs (Kirchgessner and Roth, 1978a; 1982). Improvement in feed conversion efficiency raises questions on the mode of action of organic acids such as propionic, fumaric, citric and formic acid. As has been reviewed by Kirchgessner and Roth (1988), the ergotropic effect of different organic acids may by attributed to (1) improvement in the digestibility of nutrients, (2) gastrointestinal effects, including antimicrobial effects in which potentially detrimental bacteria are destroyed by a reduction in the gastric pH and (3) changes in intermediary metabolism including energetic utilization.

The objective of the present study was to determine the effect of supplementation of propionic acid to grower diets for pigs on (1) ileal and fecal digestibilities of energy, protein, and amino acids and (2) the level of microbial metabolites in ileal and cecal digesta.

2. Materials and Methods

Six barrows (Lacombe x Yorkshire), with an average B.W. of 50 kg, were surgically fitted with a simple T-cannula at the distal ileum and in the cecum according to procedures adapted from Sauer et al. (1983) and Mosenthin (1987). Following recovery the pigs were fed one of four diets

according to an incomplete 4x4 Latin square design. The basal diet contained 15 % soybean meal, 61.9 % barley, 10 % wheat bran, 10 % sugar beet pulp, 2.6 % of a mineral- vitamin mixture and 0.5 % chromic oxide as digestibility marker. The dietary treatments were as follows:

Treatment 1: Basal diet (Control)
Treatment 2: Basal diet + 2.0 % propionic acid
Treatment 3: Basal diet + 2.5 % siliceous earth
Treatment 4: Basal diet + 2.0 % propionic acid + 2.5 % siliceous earth

Siliceous earth was supplemented to the diet as a possible adsorbent for propionic acid, which, in turn may affect the release of propionic acid during its passage in the gastrointestinal tract and thereby the site and mode of action. The diets were formulated to contain 17 to 18 % CP (% DM). The pigs were fed twice daily, 900 g each meal.

Each experimental period lasted 12 d. Following an 8-d adaptation period feces were collected for 2 d at 12-h intervals followed by a 12-h collection period of cecal digesta on d 11 and a 12-h collection period of ileal digesta on d 12. Feces and digesta were frozen immediately after sampling and pooled, where appropiate.

The pooled samples were freeze-dried, ground in a Wiley mill through a 1 mm mesh screen and thoroughly mixed prior analyses. Analyses for N and DM were conducted according to AOAC (1980) methods. Energy was measured with an adiabatic bomb calorimeter. Chromic oxide levels in feed, digesta and feces were determined according to Fenton and Fenton (1979). Amino acid analyses were performed following acid hydrolysis in 6N HCl for 24 h using an amino acid analyzer (LKB Alphatronic).

Ammonia in ileal and cecal digesta was determined photometrically using a test kit (Sigma No. 640). Analyses for cadaverine and putrescine were carried out by means of HPLC.

The results were subjected to least square analyses of variance for unequal numbers (Mehlenbacher, 1978). Least square means were compared using the Student Newman-Keuls multiple range test (Steel and Torrie, 1980).

Results and Discussion

Apparent ileal and fecal digestibilities of energy and protein were not affected ($p>.05$) by the different dietary treatments. However, the ileal digestibilities of all indispensable and dispensable amino acids were higher when propionic acid or propionic acid in combination with siliceous earth were supplemented. These differences were significant ($p<.05$) for histidine and arginine. Compared to the basal diet (control) the addition of siliceous earth decreased the digestibilities of all aminio acids. This difference was significant ($p<.05$) for valine. Compared to the diet in which 2.0 % propionic acid was supplemented, the digestibilities of most of the indispensable amino acids (arginine, histidine, isoleucine, leucine, phenylalanine, threonine, valine) and some of the dispensable amino acids (glutamic acid, serine) was lower ($p<.05$). The corresponding differences ranged from 6.8 (glutamic acid) to 12.5 (valine) percentage units. From these results can be concluded that the supplementation of propionic acid to diets of growing pigs will improve the apparent ileal digestibilities of most of the amino acids. Comparative results in the literature are not yet available. However, studies by Kirchgessner and Roth (1980) also indicate that supplementation of organic acids, such as fumaric acid, may improve fecal digestibilities of protein and energy by 2 to 3 percentage units in diets for starter pigs. There was

also a tendency for higer fecal digestibilities of protein and energy in diets for growing pigs, however this effect was not significant (Kirchgessner and Roth, 1978b). The question arises whether the fecal analysis method is sensitive enough to measure differences in protein and amino acid digestibilities resulting from supplementation of organic acids because of the modifying effect of the microflora in the large intestine.

Table 1. Effect of supplementation of propionic acid or siliceous earth or a combination of propionic acid and siliceous earth on the content of cadaverine, putrescine and ammonia in ileal and cecal digesta.

	Diets				
	Basal diet	Basal diet + prop. acid	Basal diet - + silic. earth	Basal diet + prop. acid + silic. earth	SE[a]
Ileal digesta					
Cadaverine, nmol/g DM	335.7	294.8	204.6	220.6	91.2
Putrescine, nmol/g DM	777.6	624.3	903.2	971.7	316.2
Ammonia, µg/g DM	940.9^{b}	720.4^{c}	989.4^{b}	971.7^{b}	85.5
Cecal digesta					
Cadaverine, nmol/g DM	821.0^{b}	116.5^{c}	893.8^{b}	338.6^{c}	146.9
Putrescine, nmol/g DM	2562.2^{bc}	1870.9^{cd}	2617.3^{b}	1414.0^{d}	292.9
Ammonia, µg/g DM	1037.9	879.6	1120.9	941.9	140.1

[a] Pooled standard error of the mean

[b,c,d] Means in a row with different superscripts differ ($p<.05$)

The supplementation of propionic acid decreased ($p<.05$) the level of ammonia in ileal digesta and a similar trend was observed for cadaverine and putrescine (Table 1). The addition of siliceous earth as well as the combination of propionic acid and siliceous earth did not affect ($p>.05$) the level of cadaverine, putrescine and ammonia in ileal digesta.

The supplementation of propionic acid decreased the concentrations of the microbial metabolites in cecal digesta of pigs. This difference was significant ($p<.05$) for cadaverine. The supplementation of siliceous earth had no effect on the level of amines and ammonia in cecal digesta compared to the basal diet. The supplementation of both siliceous earth and propionic acid seemed to reduce the level of amines and ammonia, which was significant ($p<.05$) for putrescine compared to the basal diet.

The results of the present study support the notion, that supplementation of pig diets with organic acids, such as propionic acid, will have a beneficial effect on the processes of digestion and absorption in the gastrointestinal tract by decreasing the formation of toxic microbial metabolites and by increasing the ileal amino acid digestibilities.

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MAIZE STARCH DIGESTIBILITY FROM CCM WITH DIFFERENT CHOPPING DEGREES AND CRUDE FIBRE CONTENTS IN ILEORECTOMIZED AND INTACT PIGS

C. Wecke & G. Gebhardt

Division of Animal Nutrition Physiology and Feed Science, Faculty of Agriculture, University of Leipzig, Germany

Abstract

Two experiments were carried out on ileorectomized and intact growing as well as on finishing pigs to estimate the digestibility of starch as the main nutrient component in corn-cob-mix (CCM) with particular consideration of its chopping degree and crude fibre content.
Independent of the live weight of the animals, the section of the digestive tract and the chopping degree and crude fibre content of the tested CCM silages were obtained corresponding very high starch digestibility values on an average of 99 % in all treatments. This results confirm, that the maize starch from CCM silage is nearly complete digested by the pig already in the small intestine.

Introduction

The aim of the experiments was to estimate the starch digestibility of CCM with particular consideration of the digestive capacity of pigs in different live weight ranges and of the chopping degree and crude fibre content of CCM silages. The concerned maize ear products were derived from maize plants of the early maturing hybrid varieties "BEMA 210" and "MUTIN". After harvesting of CCM by using combines with different sieve set equiment, grinding in hammer mills with sieves between 6 and 12 mm hole diameters and subsequent ensiling in containers or horizontale silos were get 9 CCM silage charges of various composition (s. Tab. 1). The attained chopping degree included sizes between "coarse" (46 % of the CCM particles < 2 mm) and "fine" (80 % < 2 mm). In dependence of the harvested shares of cob parts in the CCM (c. 15...75 % of the whole cob quantities) the adequate crude fibre contents (estimated by WEENDE analysis) varyed between 33 and 65 g per kg dry matter (DM). The analysis of the starch contents in the dried CCM silage charges and faeces of pigs was realized in a central laboratory of the university in Halle-Wittenberg by photometric measurement of glucose contents in the extract of the corresponding samples, wich were treated before with perchloric acid ($HClO_4$) and by subsequent multiplication of these values with adequate conversion factors. In the result of this investigations were get values between 50 and 54 % starch in the DM of the tested CCM silages. These data are in good agreement with some literature values (Holmes et al. 1973, Schneider & Kirchgessner 1978).

Table 1. Characteristics of the tested CCM silages.

Trial	Treatment	Chopping degree	Sieve holes mm[1)]	Particles < 2 mm %	Cob share[2)]	Crude fibre g/kgDM	Starch g/kgDM
1	A	coarse	12	46,0	high (75%)	65	496
	B	medium	10	54,6			
	C	fine	8	64,4			
2	D	medium	10	62,9	high (75%)	63	496
	E	fine	6	79,7			
	F	medium	10	59,8	medium (45%)	48	517
	G	fine	6	78,3			
	H	medium	10	56,6	low (15%)	33	538
	I	fine	6	76,9			

1) Hole diameters of the hammer mill sieves
2) Shares of cobs in the CCM in relation to the whole cob quantities

The majority of the newer values of starch contents in CCM can be placed however over this analysed results (for instance: Burgstaller et al. 1984, DLG 1984, Roth-Maier & Kirchgessner 1984).
For the determination of the starch digestibility were carried out two digestive experiments. In trial 1 were used castrated male pigs in a live weight range from 95...105 kg and CCM silages with 3 different chopping degrees, in trial 2 intact as well as ileorectomized female pigs with live weights between 30 and 50 kg and CCM silages with 2 different shopping degrees and 3 different cob shares in a 2x3 factorial procedure.
All experimental data were interpreted by the help of the 2-factorial variance analysis.

Results and Discussion

Between intact growing pigs in a live weight range of circa 40 kg and finishing pigs with an average of 100 kg live weight could´nt found significant differences in the digestibility of maize starch from the tested different CCM silages (Tab. 2). Independent of the live weight of pigs and the qualitative composition of the CCM charges were obtained digestibility values over 99 %.
The results about the influence of chopping degrees on starch digestibility also indicate no effect, if more than 50 % of the CCM particles are smaller than 2 mm (Tab. 3). This is reached by using hammer mill sieves with hole diameters of 10 mm. The mean value in treatment A (98,63 %),

Table 2. Influence of pigs live weight on starch digestibility.

CCM silage		Treatment	n	live weight of pigs	
Chopping degree	Cob share			c. 100 kg (Trial 1)	c. 40 kg (Trial 2)
				starch digestibility (%)	
medium	75 %	BD	9	99,27 ±0,03	99,18 ±0,14
medium	different	BDFH	17	99,27 ±0,03	99,29 ±0,14
medium -fine	different	B-I	33	99,30 ±0,05	99,39 ±0,09

in which only 46 % of the particles were < 2 mm, was significantly lower ($\alpha < 0,05$) than all the other digestibility values obtained at the end of the digestive tract, though only 0,6...0,9 % relatively. For that reason the investigated starch digestibilities are good to arrange in the results of crude nutrient digestibilities of different grinded CCM silages (Roth-Maier & Kirchgessner 1984, Wecke et al. 1989).

The increased crude fibre contents in the CCM silages has affected the ileal (praecaecal) starch digestibility positively ($\alpha < 0,05$), but likewise to a very small size (0,8 %). On the other hand, contrary to these results, was observed a tendentious decrease in the faecal starch digestibilities of the tested CCM silages under the same conditions. After addition of the starch digestibilities of both places of digestion were get in each case equal mean values, independent of the crude fibre contents or cob shares in the CCM silages. This results are at last in good agreement with findings of Graham et al. (1985) and Metz et al. (1985). A comparison of the mean values of ileal with the faecal starch digestibilities showes a significant superiority ($\alpha < 0,05$) of the latter digestibility values. Doubtless this result proceedes first of all from the very small standard deviations of bouth mean values. Therefore it should in no case be overrated, since the faecal starch digestibilities are higher by only 0,4 % relatively.

The presented investigations confirm, that the maize starch from CCM silages is complete digested by the pig. The very high starch digestibilities of 99 % good agree with results from literature. Investigations on pigs with CCM silages (Roth-Maier & Kirchgessner 1984) and maize corn (Sugimoto & Takahashi 1986) have also supplied values between 98 and 100 %. Finally, it can be going out as well from the results of the presented digestive trial with ileorectomized pigs as from literature values (Holmes et al. 1973), that the maize starch from CCM and corn is digested

Table 3. Ileal and faecal starch digestibility of different CCM silages.

Trial	Treatment	Sieve holes mm	Particles < 2 mm %	Cob share %	Crude fibre g/kg DM	n	Starch digestibility %		
							Ileal	Faecal	Mean
1	A	12	46	75	65	5		98,63 ±0,25	
1	B	10	55	75	65	5		99,27 ±0,03	
1	C	8	64	75	65	5		99,32 ±0,10	
2	DFH	10	60	75...15	63...33	21	98,83 ±0,22	99,29 ±0,14	99,09 ±0,13
2	EGI	6	78	75...15	63...33	21	99,08 ±0,14	99,49 ±0,11	99,29 ±0,10
1	ABC	8...12	46...64	75	65	15		99,07 ±0,12	
2	DE	10/6	63/80	75	63	14	99,41 ±0,05	99,13 ±0,10	99,27 ±0,07
2	FG	10/6	60/78	45	48	14	98,75 ±0,24	99,48 ±0,11	99,17 ±0,15
2	HI	10/6	57/77	15	33	14	98,64 ±0,24	99,52 ±0,20	99,14 ±0,19
Mean							98,96 ±0,13	99,39 ±0,09	

nearly complete already in the small intestine of growing pigs. The microbial starch digestion in the colon with its negative effect on the energetic utilization, like observed for instance with unheated potatoes, is no object accordingly in the case of CCM silage.

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DIGESTIBILITY OF CRUDE FIBRE AND NONSTARCH POLYSACCHARIDES FROM RYE AND TRITICALE IN PIGLETS

Rosemarie Köhler & F. Reinisch

Division of Animal Nutrition Physiology and Feed Science,
Faculty of Agriculture, University of Leipzig, Germany

Abstract

The digestibility of crude fibre, NDF, ADF, cellulose, hemicellulose and lignin in rye and triticale was estimated on piglets. The digestibility of NDF was much higher (52,7 % in rye and 50,1 % in triticale) than the crude fibre digestibility, which amounted to 36,2 % in rye and to 0 % in triticale. There was no significant influence of conservation of the faeces with 5 percent sulfuric acid on the digestibility of dietary fibre with exception of the hemicellulose digestibility of rye.
Keywords: digestibility, piglet, dietary fibre, rye, triticale

Introduction

A well known fact is, that the values of crude fibre, obtained by the WEENDE analysis, don t meet the content of fibre in the feedstuffs. The same problem is to taken into consideration for the digestibility of crude fibre. More certainly in this case is the use of the dietary fibre concept (van Soest 1967). As pointed out by Robertson and van Soest (1981) the faecal bacterious cell wall substances are soluble in neutral and acid detergents and consequently they can not falsify the results based on the detergent methods. Using these two different methods the digestibility of crude fibre and dietary fibre in grains of rye and triticale should be determined.

Materials and Methods

Twelve piglets, weighing about 20 kg, were randomly assigned to two experimental groups. The first group received a rye diet, the second a diet which contained triticale as the main component. Each of the two diets contained 96 % of grain, 1,5 % of mineralic mixture, 1% of vitamine mixture and 1,5 % of oil. The content of nutrients in the grains is presented in table 1. The animals were kept in metabolic cages. The food was given three times daily. During the main experimental period of 4 days the faeces were sampled twice daily. The faeces of three piglets of each group were pooled with 5 percent sulfuric acid before deepfreezing, whilst the remaining samples of faeces were freezed without acid. Diet and faeces were analysed for crude fibre, crude protein, fat and ash. The cell wall substances NDF (neutral detergent fibre), ADF (acid detergent fibre), cellulose, hemicellulose and lignin were estimated by the detergent extraction method (Goering & van Soest 1970, Strutz & Becker 1985).

Table 1. Content of nutrients (% of dry matter) in grains of rye and triticale.

	Rye "Pluto"	Triticale "Grado"
Crude protein	11,4	15,0
Crude fibre	3,4	4,6
Fat	1,2	1,2
Ash	2,0	3,0
NDF	15,2	15,5
ADF	3,1	4,8
Cellulose	2,5	4,0
Lignin	0,6	0,8
Hemicellulose	12,2	10,7

Results and Discussion

In table 2 the digestibility values of nutrients and dietary fibre are summarized. Because the differences in digestibility between conserving the samples of faeces with or without acid with only one exception were not significant, all the values of each group (6 piglets) could be used for the mean value. The only significant difference dependent on sampling of faeces with or without acid was between the digestibilities of hemicellulose in the rye, which amounted to 58,2 % respectively 61,2 %. General the results tended to be higher in the samples preserved without acid.

Moreover there were considerable differences in the dry matter of faeces between the piglets of the two groups. The higher values of dry matter were observed in the triticale group: 46,4 % $\pm$ 3,7 without and 22,8 % $\pm$ 0,6 with acid conservation. Against this the values of dry matter were in the faeces of piglets of the rye group: 32,5 % $\pm$ 3,4 without and 18,2 $\pm$ 1,5 with acid. These results establish the well known fact of raising the moisture content of the faeces from young animals by rye feeding.

The digestibility of total cell wall substances (NDF) in rye and triticale is much higher than the digestibility of crude fibre. In the rye the crude fibre digestibility is lying between digestibility values of ADF and cellulose. In triticale the digestibility of

Table 2. Digestibility of dietary fibre (%) in grains of rye and triticale.

Digestibility of	Rye "Pluto"	Triticale "Grado"
Crude fibre	36,2 $\pm$ 4,2	(12,5 $\pm$ 14,1)
NDF	52,7 $\pm$ 1,3	50,1 $\pm$ 8,6
ADF	28,0 $\pm$ 2,1	33,3 $\pm$ 9,9
Cellulose	43,7 $\pm$ 1,7	44,0 $\pm$ 9,4
Lignin	2,0 $\pm$ 4,2	10,1 $\pm$ 13,2
Hemicellulose	59,8 $\pm$ 1,7	57,0 $\pm$ 8,4

crude fibre amounted to zero (exactly to -12,5 %), whilst ADF and cellulose with regard to digestibility differs only few from the values in rye.

There is no explanation for the very low digestibility of crude fibre in the triticale. Maybe this result is influenced by the age of the animals because in the feed value tables of DLG (1984) the digestibility of crude fibre in triticale for adult pigs amounts to 35 %. Also the digestibility of lignin in triticale is not real. As already pointed out by van Soest (1983) higher lignin digestibilities than zero are caused by analytical problems of recovery of low lignin amounts in the faeces.

Summarizing these results is to conclude, that the piglet can digest the dietary fibre in rye and triticale to about 50 % and that the crude fibre is not able to give the right values of digestibility.

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EFFECTS OF A PROPIONIC ACID CONTAINING FEED ADDITIVE ON PERFORMANCE AND INTESTINAL MICROBIAL FERMENTATION OF THE WEANLING PIG[1]

A. G. Mathew, A. L. Sutton, A.B. Scheidt, D.M. Forsyth, J.A. Patterson and D.T. Kelly

Department of Animal Sciences and Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, Indiana USA 47907.

Abstract

Two experiments were conducted to determine the effects of Luprosil-NC® (L) (propionic acid, 53.5%; ammonium hydroxide 9.5%; 1,2 propanediol, 11.5%; water, 25.5%: BASF Corp.) on performance and enteric microbial populations of the young pig. In Experiment I, 200 weanling pigs were allotted to five treatment diets (0%, .25%, .5%, 1.0% L and .125% tylan-sulfa (TS). Five pigs from each treatment were sacrificed on the 4th week and the 8th week of the trial to collect digesta samples from the stomach, duodenum, cecum, proximal and distal colon. Pig gains and efficiencies were improved ($p<.03$) when fed the TS diet and the .25% L during the nursery phase. At the eighth week sampling, pigs fed .25% L and TS had higher ($p<.02$) Lactobacillus concentrations in the stomach and pigs fed the control had lower ($p<.05$) Lactobacillus concentrations in the duodenum compared to the other treatments.

In the second experiment, two trials were conducted with six 21-day old pigs (twelve total) fitted with t-cannulas in the proximal ileum. At weaning, pigs were randomly assigned to 3 diets (0, .5% and 1.0% L). Digesta samples were obtained on days 0, 2, 4, 6, 8, 11 and 14 after weaning. All pigs showed a significant ($p<.10$) reduction in Lactobacillus and a corresponding increase in E. coli concentration on day 2. There was no difference between diets at the $\alpha=.05$ level.

Introduction

A major economic problem in swine production is the occurrence of digestive disorders, such as colibacillosis and diarrhea, caused by microbial abnormalities in the lower gastrointestinal (GI) tract of pigs. Often during the stressful time of weaning, pigs have reduced body weight gains, poor feed intake, appear lethargic and fail to regain normal health and performance (Kohler and Moon, 1984).

Potential causes of these disorders are lack of proper sanitation, reduced immunity, nutritional imbalances or poor quality feeds (molds, toxins, etc.), environmental stress (high humidity, improper ventilation, etc.) and infectious agents (Kohler and Moon, 1984). The effects of antibiotics, pH, nitrogen sources and levels, carbohydrate sources, fiber content and volatile fatty acids (VFA) on diarrhea and the E. coli concentrations in the intestine have been investigated (Dierick, et al., 1986; Fevrier and Aumaitre, 1979; Kim, et al., 1978; Muralidhara, et al, 1977; Prohaszka and Baron, 1982; Prohaszka and Lucas, 1984; Fevrier, 1978). It appears that the type of microbial metabolism in the intestine resulting in different proportions of specific end products, i.e. volatile fatty acids, ammonia, ionized cations, can greatly affect the specific microbial populations that predominate. If conditions exist in the intestinal lumen that support microbial populations which will be competitive with and predominate over pathogens, then resistance to enteric diseases will be enhanced.

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Addition of organic acids or salts have been proposed to alter pH and VFA's of the GI contents in the pig. Organic acids (propionic acid or propionic-acetic acid combinations) have been shown to preserve high moisture feed ingredients used for livestock and poultry diets by controlling mold development and reducing bacterial growth. Luprosil-NC® is a product containing propionic acid, ammonium hydroxide, water, and 1,2, propanediol which is non-corrosive to metal equipment, serves as a diet preservative, and has the potential of altering the microbial ecology within the digestive system of the pig. However, research is needed to understand the interrelationships between this product and the microbial populations, competition for nutrients by the host animal and microbes, and the control of harmful microbes for improved health of the animal. Therefore, the objective of the research studies reported here, was to determine the effect of Luprosil-NC® on performance, selected microflora populations and intestinal fermentation patterns of growing pigs.

Materials and Methods

Experiment I

Two hundred weanling pigs were used in a nursery-grower trial. The pigs were assigned to five dietary treatments (0, .25%, .5%, 1.0% Luprosil-NC® and .125% tylan - sulfa) with five replicate pens of each experimental diet with eight (8) pigs per pen.

Pigs were weaned at four (4) weeks of age without prior creep feeding and placed in raised nursery decks. Five pigs per treatment (one from each pen; 25 pigs total) were euthanatized at the fourth week of the trial for sampling of intestinal contents. The rest of the pigs were moved from the nursery to the growing facility, keeping pigs from all pens together for an additional four week feeding trial. An additional pig from each pen (five per treatment; 25 pigs total) was euthanatized at the end of the four week growth phase for sampling of intestinal contents.

Gastrointestinal contents from the stomach, upper portion of the duodenum, cecum, and proximal and distal colon were collected immediately after the pigs were euthanatized. Gastrointestinal samples were assayed for E. coli using Bacto EMB agar, and Lactobacillus using Bacto Rogosa SL agar. Volatile fatty acids were also determined on gastrointestinal contents using gas chromatography methods.

Experiment II

Two trials were conducted with six 21-day old pigs (12 total). All pigs were surgically fitted with t-cannulas in the proximal ileum 7 days prior to weaning. After a recovery period of 7 days, the pigs were weaned and assigned to 3 experimental diets (control, .5% and 1.0% Luprosil-NC®). No feed antibiotics were used. The digesta of each pig was sampled on days 0, 2, 4, 6, 8, 11 and 14 after weaning. Samples were analyzed for E. coli, Lactobacillus and pH.

Results and Discussion

Experiment I

Average daily gain (ADG) and feed efficiency (FE) were superior ($p<.03$) for the pigs fed the .25% Luprosil-NC® diet and antibiotic (tylan-sulfa) compared to the other dietary treatments during the nursery phase (0-4 wks) of the experiment. ADG was improved by 10.3% and 14.7% respectively over the control diet and FE was improved 11.3% and 13.3% respectively over the control diet in that feeding period.

In the 8 week old pigs (4th week on trial), Lactobacillus colony counts were highest in the cecum and large intestine. In general, the Luprosil-NC® diets tended to promote this growth in the lower GI tract. In the 12 week old pig (8th week on trial), Lactobacillus concentrations increased throughout the GI tract, but there were significant effects of Luprosil-NC® and the antibiotic diets in the stomach and small intestine (Table 1). Pigs fed the Luprosil-NC® and antibiotic diets increased ($p<.05$) Lactobacillus concentrations in the duodenum. In the

stomach, pigs fed the .25% Luprosil-NC® and the antibiotic diets had higher Lactobacillus concentrations than those fed the control diet. There were no significant differences in E. coli concentrations between diets (Table 2).

Table 1. Effect of Luprosil-NC® on Lactobacillus in intestinal contents (colonies per gram)[b] (Exp I).

Time of Sampling	Location	C[a]	Luprosil - NC® % .25	.5	1.00	A[a]
4th wk	Stomach ($x10^5$)	.66	.46	5.21	4.00	3.08
	Duodenum ($x10^5$)	5.88	0.00	6.76	.45	2.54
	Cecum ($x10^7$)	5.66	8.42	12.12	9.31	5.46
	Proximal LI ($x10^7$)	10.18	15.96	25.46	3.83	12.21
	Distal LI ($x10^7$)	9.22	26.17	22.37	14.22	11.32
8th wk	Stomach ($x10^6$)[d]	2.78	31.61	19.79	21.43	32.53
	Duodenum ($x10^6$)[c]	2.27	18.98	19.37	11.33	31.57
	Cecum ($x10^8$)	6.32	5.50	1.35	4.47	4.68
	Proximal LI ($x10^8$)	7.96	10.68	6.10	7.49	8.88
	Distal LI ($x10^8$)	10.12	7.00	8.00	9.08	9.83

[a] C= corn-soy basal diet w/o antibiotic; A = corn-soy basal diet with .125% tylan-sulfa.
[b] $p < .11$ for dietary treatment effect in all contents in 8th week, $p < .0001$ for location effect in both sampling periods. SEM = 4.5×10^5.
[c] $p < .05$, control < all other dietary treatments.
[d] $p < .02$, .25% Luprosil-NC®, antibiotic > control.

Table 2. Effect of Luprosil-NC® on E. coli in intestinal contents, (colonies per gram)[b] (Exp I).

Time of Sampling	Location	C[a]	Luprosil - NC® % .25	.5	1.00	A[a]
4th wk	Stomach ($x10^6$)	5.57	4.48	11.70	7.49	16.30
	Duodenum ($x10^6$)	11.00	3.62	14.39	27.67	37.75
	Cecum ($x10^6$)	24.75	5.22	6.12	10.19	17.32
	Proximal LI ($x10^6$)	30.56	5.67	9.20	4.12	14.82
	Distal LI ($x10^6$)	12.13	3.12	51.03	7.85	32.69
8th wk	Stomach ($x10^5$)	8.40	7.01	11.39	.94	3.42
	Duodenum ($x10^6$)	2.75	.42	2.36	.59	.33
	Cecum ($x10^6$)	9.17	12.63	5.80	4.21	62.16
	Proximal LI ($x10^6$)	21.27	.29	7.94	2.83	24.30
	Distal LI ($x10^6$)	17.17	20.75	7.29	2.90	10.38

[a] C= corn-soy basal diet w/o antibiotic; A = corn-soy basal diet with .125% tylan-sulfa.

Volatile fatty acids of intestinal contents of the pigs were similar in concentration between the experimental diets. There were differences at the 8th week sampling ($p<.10$) with a higher proportion (%) of butyrate, and valerate, but a lower percent of acetate, isobutyrate and isovalerate in cecal and stomach contents of pigs fed the .25% Luprosil-NC® diet as compared to the control (Table 3).

Table 3. Effect of Luprosil-NC® on volatile fatty acids in cecal contents (mMol/L) (Exp I).

			Lurposil-NC® %				
Time of Sampling	Location	C[a]	.25	.5	1.00	A[a]	SEM
4th wk	Acetate	80.2	67.7	67.5	54.0	80.9	5.1
	Propionate	44.4	37.0	39.6	33.8	42.7	2.1
	Butyrate	22.4	19.7	23.6	24.0	23.1	1.6
	Isobutyrate	0.5	0.4	0.4	0.5	0.7	.1
	Valerate	4.8	4.1	5.6	5.1	6.1	.5
	Iso-valerate	4.7	0.3	0.4	0.6	0.7	.5
8th wk	Acetate	83.2	76.8	69.5	99.7	64.6	6.3
	Propionate	40.5	46.9	59.1	48.6	36.3	3.0
	Butyrate	20.2	27.0	22.4	34.1	28.8	1.6
	Iso-butyrate	1.0	0.4	0.6	0.8	0.5	.1
	Valerate	4.3	7.4	4.0	6.6	9.2	.5
	Iso-valerate	2.0	1.4	1.8	1.7	1.2	.2

[a] C= corn soy basal diet w/o antibiotic; A = corn soy basal diet with .125% tylan-sulfa.

Experiment II:

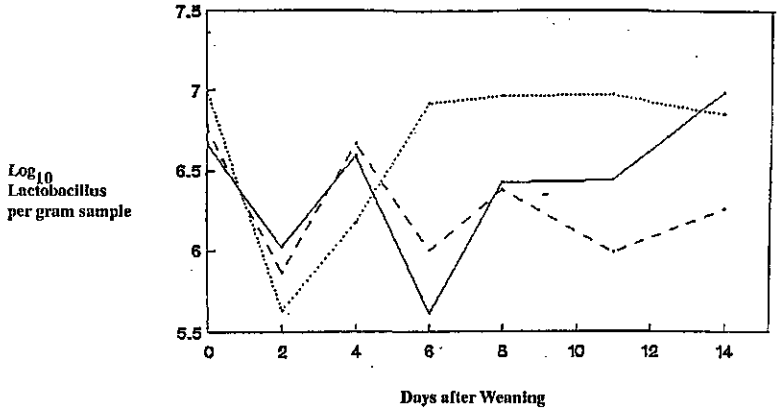
All pigs showed a significant decrease ($p<.10$) in Lactobacillus on day 2, when a corresponding increase in E. coli occurred (Figures 1 and 2). This was followed by a recovery of Lactobacillus to near preweaning concentrations over the next several days and a decline in E. coli. E. coli concentrations were significantly lower ($p<.10$) for the 1% Luprosil-NC® treatment over the two week trial period. There were no differences in pH between diets at the $\alpha=.10$ level. The reason for the decrease in Lactobacillus soon after weaning is not clear. It may be related to the reduced intake which often occurs the first few days post weaning. Additionally, Lactobacillus may require a longer adaptation period to the change in nutrient sources i.e., sow's milk to dry feed, which occurs at weaning. This may give a competitive advantage to E. coli in the gastrointestinal tract of the newly weaned pig.

Conclusions

Low levels of Luprosil-NC® in weanling pig diets supported efficient feed utilization and growth especially during the first four weeks after weaning. There were trends toward reduced E. coli concentrations and increased Lactobacillus concentrations in upper portions of the intestinal contents of pigs fed diets with Luprosil-NC® additions.

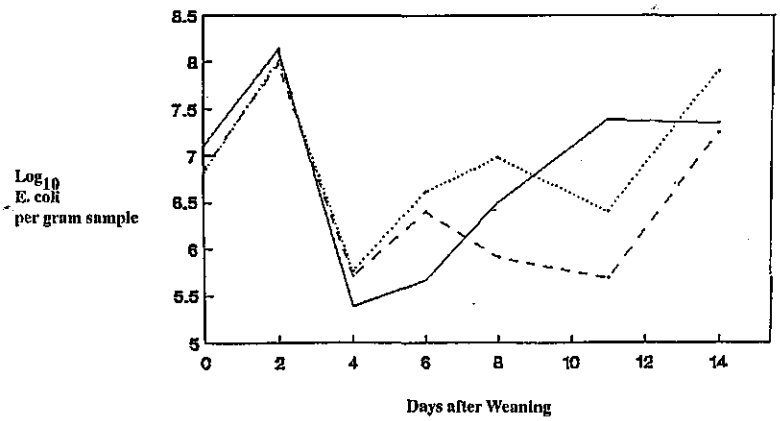
Abrupt changes in Lactobacillus and E. coli can occur during the stress of weaning. Luprosil-NC® did not significantly alter volatile fatty acid concentrations of intestinal contents.

EFFECT OF LUPROSIL-NC® ON ILEAL LACTOBACILLUS CONCENTRATION



Control = solid line, .5% L = dotted line, 1.0% = broken line
Standard error = .39

EFFECT OF LUPROSIL-NC® ON ILEAL E. COLI CONCENTRATION



Control = solid line, .5% L = dotted line, 1.0% = broken line
Standard error = .38

Additional intensive research studies are needed to further determine more clearly the mode of action and role of Luprosil-NC® on the microbial ecology in the GI tract of the pig.

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THE EFFECT OF THE CARBOHYDRATE FRACTION FROM VARIOUS RAW MATERIALS ON THE PERFORMANCE, THE ILEAL AND FAECAL DIGESTIBILITIES AND THE ENERGETIC VALUE OF THE DIET

C.H.M. Smits, W.A.G. Veen, G.J. Borggreve, J.J. Heeres-Van der Tol

CLO-Institute for Animal Nutrition "De Schothorst", Lelystad, the Netherlands

Abstract

Three experiments were conducted to investigate the effect of diets varying in source and amounts of NFE and crude fibre on the performance, the ileal and the faecal digestibilities and the energetic value.
In the growth trial the diets with a low proportion of starch in the NFE and a high crude fibre content gave on average a 4,5% lower weight gain and a 3% higher feed conversion than the control diet with the highest proportion of starch in the NFE. The meatpercentage in the carcasses of the pigs on the diets high in crude fibre and NFE minus starch was on average 1,5% higher indicating a lower fat deposition. The addition of a relatively inert crude fibre and NFE source (straw and sunflowerseedhulls) did not have a negative influence on the performance of the pigs. The digestibility experiments showed that of the dNFE fraction more than 80% was digested at the end of ileum in the control diet with 45% of starch. Of the diets based on grain byprodycts, oilseedmeals/expellers and pulps only 62 to 72% of the NFE fraction was digested at the end of the ileum. A greater part of the NFE of these diets must have been fermented compared to the control diet. It is concluded that the estimation of the Rostock formula of the energetic value of diets is inaccurate for diets rich in dNFE minus starch and crude fibre.

Introduction

The energetic value of the raw materials of pig diets in the Netherlands is calculated with the Rostock formula (Nehring, 1967): NE_{pigs} = 2.59 x dcprotein + 8,63 x dcfat + 1,50 x dcfibre + 3,03 x dNFE fraction (Nitrogen Free Extract). For the NFE fraction the formula does not distinguish the carbohydrate fraction that is digested by enzymes produced by the animal itself and the fraction that is fermented by the microbes. This is important for the energetic value of the diet. The end products of the praecaecal digestion of carbohydrates are monomeric sugars. During the digestion steps the energy losses are relatively low. The end products of the fermentation of carbohydrates are volatile fatty acids which have a lower NE value than glucose. Furthermore energy losses will occur by the production of heat and methane (Muller and Kirchgessner, 1986).
Highly fermentable but non digestible carbohydrates are non starch polysaccharides and α-galactosides like pectins, ß-glucans, pentosans, stachyose and raffinose. The main carbohydrate fraction in pig diets that is digested by animal produced enzymes is starch. Muller and Kirchgessner

Table 1 Composition of the diets

Content (%)	A 45% starch	B grain by-products	C oilseed-meals/ expellers	D pulps	G A incl. pulps and soybean-hulls
Feedstuff					
citruspulp	-	-	-	6	4,7
animal fat	-	3,85	3,35	3,6	0,85
peas	5	5	5	5	4,42
barley	5	5	5	5	4,7
rapeseed oilmeal	-	-	2,5	-	-
hominy feed	-	9,6	9,55	-	-
maize gluten feed	-	10	-	-	-
molasses, cane	2,8	2,8	2,8	2,8	3,75
maize	12,9	-	-	-	12,12
maize gluten meal	1,5	-	-	1,5	1,41
beetpulp	-	-	-	6	5,64
palmkernel, exp.	-	-	2,5	-	-
wheat middlings	-	15	-	-	-
tapioca meal	34,3	25	30,5	33,3	18,87
wheat	13,6	-	-	-	12,73
groundnut, exp.	-	-	2,7	-	-
coconut, exp.	-	-	2,5	-	-
soybeanhulls	-	0,9	-	10,6	10,15
soybean oilmeal	21,9	19,5	15,4	22,6	16,53
sunflower oilmeal	-	-	15	-	-
limestone	0,2	0,7	0,5	-	-
monocalciumphosphate	0,5	0,4	0,4	0,7	0,65
Ca-propionate	0,5	0,5	0,5	0,5	0,47
premix	0,5	0,5	0,5	1,35	0,84
salt	0,3	0,25	0,3	0,25	0,23
diamol	1	1	1	1	0,94
Total	100,00	100,00	100,00	100,00	100,00

Calculated contents*	A	B	C	D	G
energy content(kcalNE/kg)	2197	2197	2195	2195	2077
NFE (gr/kg)	591	526	492	518	549
starch (gr/kg)	447	307	297	288	345
NFE minus starch (gr/kg)	144	219	195	230	214
crude fibre (gr/kg)	30	47	69	73	67
cellulose** (gr/kg)	29	36	56	70	66
NCP** (gr/kg)	103	143	120	124	136

* All diets contained 7.3 grammes/kg digestible lysine
** Analysed contents

(1989) have calculated that the efficiency of utilization of energy via hindgut fermentation compared to that of praecaecally digested starch was about 70%. The hypothesis is that feedstuffs with a low starch content and a high content of non digestible but fermentable NFE are overestimated in energy content by the Rostock formula and/or feedstuffs with a high starch content are underestimated.
Another disadvantage of diets rich in non starch NFE and crude fibre is that they can have an influence on the digestion of crude fat, crude protein and minerals (Graham et al, 1986; Laplace et al, 1989; Metz, 1985; Stanogias and Pearce, 1985). Especially the fat digestion seems to be depressed by the non starch carbohydrate fraction (Drochner, 1984; Just et al, 1980).

Experimental procedure

Three experiments were conducted to study the effect of the amount and nature of NFE and crude fibre on the performance, the energetic value and the ileal and faecal digestibilities. Seven diets were formulated containing different levels and sources of carbohydrates and dietary fibre. The positive control diet (A) contained 45% starch and 3% crude fibre and was based on tapiocameal and wheat as main carbohydrate sources. The diets B, C and D were formulated to contain the same net energy content according to the Rostock formula. In these diets starch was exchanged for fat and crude fibre with different raw materials. In treatment E 30 grammes starch was added to each kg of diet C in order to get the same net energy content in the diet as treatment A when the diets were calculated according to a modified Rostock-formula (Borggreve et al, 1976). To each kg of diet A 35 grammes of straw and 51 grammes of sunflowerseedhulls was added to obtain diet F with the aim to study the influence of a relatively inert crude fibre source. Diet G was based on the composition of diet A but had a 4% higher crude fibre content with beetpulp, citruspulp and soybeanhulls as crude fibre sources. The composition of the diets are given in table 1. The diets A, B, C, D, E and F were used in the performance trial. In this experiment 96 individually housed pigs were allocated to the diets with each group of 16 animals consisting of 8 barrows and 8 sows. The experimental diets were given from 38 to 107 kg live weight.
The animals were scale fed during the preliminary and the experimental period. The intake was expected to be 90% of the ad libitum feed intake.
In the first digestibility experiment the diets A, B, C and D were used and in the second trial the diets A, F and G. Ileorectostomised pigs were used for the determination of the ileal digestibilities. The pigs were placed in metabolism crates and fed twice daily. The feeding level was 4% of their live weight. The initial weight was on average 30 kg. During the collection period there were no feed refusals.

Results and Discussion

The performance data, corrected for differences in dressing percentage, are given in table 2. The diets B, C and D resulted in lower weight gains than diet A resp. -4.6, -4.1 and -5.3%. These differences cannot be explained by the differences in energy intake according to the unadapted Rostock formula i.e. resp. -1.5, -1.5 and -0.8%. The data seem to confirm the hypothesis that the formula overestimates the energy content of the diets rich in NFE minus starch and a high fat content. The results of diet E were expected to be better than the results of diet C because in addition to each kg of diet C 30 grammes of cornstarch was added to obtain diet E.

Table 2 The effect of the amount and source of dietary fibre on the performance and carcass quality of pigs

Treatment	A 45% starch	B grain-bypro-ducts	C oilseed-meals/ expellers	D pulps	E C+add. corn starch	F A+add. straw and sunflower-seedhulls	LSD (0,05)
feed supply(%)	100	100	100	100	103	108	
38-107,kg							
growth* (gr/day)	912	872	876	866	881	918	37
feedintake (kg/day)	2,50	2,48	2,50	2,49	2,56	2,68	0,04
energy intake** (Mcal.NE/day)	5,59	5,50	5,50	5,54	5,63	5,67	0,09
feed conversion	2,74	2,84	2,85	2,88	2,91	2,92	0,11
energy conversion (Mcal.NE/kg growth)	6,13	6,31	6,28	6,43	6,39	6,18	0,24
dressing, %	75,4	74,8	74,0	73,9	75,0	75,3	0,8
carcass meat, %	52,5	53,9	54,5	53,8	53,5	53,8	1,4

* growth corrected for differences in dressing percentage
** energy content corrected for differences between calculated and analysed contents

No explanation can be given for the results of this treatment.
The addition of 8% straw and sunflowerseedhulls to diet A (diet F) did not have a negative influence on the weight gain or on the energy conversion. Thus the addition of praecaecal undigestible and poorly fermentable carbohydrates did not affect the energetic value of the original diet. Diet A gave a significant lower meatpercentage of the carcasses than the diets B, C and D. The lower average meatpercentage of the carcasses of treatment A indicate a higher fat deposition than by treatments B, C and D.

The results of the two digestibility trials are given in table 3. The mean digestibility coefficients for diet A were lower in the first experiment than in the second experiment. In the second experiment the same ileorectostomised pigs were several weeks older. Therefore, it is only possible to compare the results of the treatments within each trial. The calculated crude protein digestibility according to the feedstuffs table (CVB, 1988) is also given in table 3.
The crude protein and crude fat digestibility of diet A was as expected higher than for the other diets. There was however a discrepancy between the calculated digestibilities and the measured digestibilities. This can be explained by differences in the techniques used and age or weight of the animals.

Remarkably low ileal digestibilities of crude protein, crude fat and NFE were found for the diets C and D based on resp. oilseedmeals/expellers and pulps/soybeanhulls. These two diets tended to give also lower ileal starch digestibilities than diet A. The digestibility of the NFE minus starch at the end of the ileum was on average 21% and at the end of the intestinal tract 77%. Especially the diet with the pulps (D) seemed to be fermented intensive in the caecum and the colon. The experiment shows very clearly that the starch content is digested almost completely in the ileum and that the NFE minus starch is fermented to a large extent in the caecum and the colon. In diet A and F approximately 84% of the digested NFE was digested

Table 3 The influence of the amount and nature of NFE and crude fibre in the diets on the ileal and faecal digestibilities .

	crude protein faecal determined	crude protein faecal table	crude protein ileal determined	crude protein ileal table	crude fat faecal determined	crude fat ileal determined
I						
A	79,8 ± 4,3	(86,9)	66,7 ± 3,4	(76,0)	69,4 ± 3,2	72,2 ± 1,5
B	73,4 ± 5,1	(82,5)	55,0 ± 5,6	(73,3)	73,9 ± 4,6	63,4 ± 4,3
C	72,6 ± 2,0	(82,6)	45,0 ± 10,3	(69,3)	74,1 ± 5,0	64,1 ± 6,9
D	69,5 ± 2,9	(81,9)	46,2 ± 17,1	(69,9)	71,6 ± 3,3	50,2 ± 12,9
II						
A	81,7 ± 1,9		78,6 ± 1,4		71,3 ± 1,6	75,5 ± 3,3
F	79,4 ± 1,6		74,4 ± 4,6		71,5 ± 2,4	70,6 ± 5,1
G	73,3 ± 2,8		63,6 ± 6,0		68,2 ± 1,4	69,5 ± 4,2

	crude fibre faecal	crude fibre ileal	starch faecal	starch ileal
I				
A	47,6 ± 6,6	0	99,8 ± 0,09	98,7 ± 0,5
B	44,0 ± 3,9	0	99,7 ± 0,02	97,4 ± 1,1
C	37,7 ± 3,0	0	99,5 ± 0,2	96,4 ± 0,5
D	44,1 ± 10,4	0	99,5 ± 0,3	96,2 ± 0,8
II				
A	45,6 ± 3,6	0	99,8 ± 0,09	98,8 ± 0,1
F	22,1 ± 3,1	0	99,8 ± 0,07	98,6 ± 0,7
G	34,7 ± 2,7	0	99,8 ± 0,1	97,3 ± 0,7

	NFE faecal	NFE ileal	NFE minus starch faecal	NFE minus starch ileal
I				
A	93,2 ± 0,9	76,8 ± 0,4	78,2 ± 2,9	27,2 ± 0,3
B	87,4 ± 0,3	63,1 ± 1,8	73,1 ± 0,7	22,9 ± 4,8
C	88,4 ± 0,8	61,9 ± 3,6	75,3 ± 1,6	21,3 ± 7,4
D	90,7 ± 0,6	56,2 ± 8,2	80,8 ± 1,1	11,2 ± 17,0
II				
A	92,6 ± 0,1	78,3 ± 0,5	76,4 ± 0,4	31,9 ± 1,8
F	89,2 ± 0,1	74,5 ± 1,6	66,9 ± 0,2	24,1 ± 3,6
G	89,9 ± 0,3	63,7 ± 2,2	77,5 ± 0,6	21,3 ± 5,2

	NCP* faecal	NCP* ileal	cellulose faecal	cellulose ileal
I				
A	71,5 ± 1,7	25,4 ± 4,3	59,0 ± 10,5	0
B	64,4 ± 1,7	6,3 ± 5,5	36,0 ± 8,1	0
C	65,0 ± 1,5	0	45,4 ± 11,9	0
D	76,7 ± 1,1	0	42,8 ± 16,8	0
II				
A	71,8 ± 2,2	31,6 ± 2,4	54,9 ± 9,8	6,4 ± 12,6
F	59,9 ± 1,7	18,9 ± 7,4	33,7 ± 4,1	0
G	72,1 ± 1,2	14,0 ± 2,7	28,0 ± 3,7	0

* Non Cellulose Polysaccharides

praecaelly, whereas for the diets B, C, D and G this was between 62 and 72%.
At the end of the intestinal tract on average 69% of the NCP fraction and 46% of the cellulose had been digested in experiment I.
The NCP and cellulose contents were determined because these fractions are chemically better defined than the crude fibre fraction (Englyst and Cummings, 1988). The digestibilities of the different carbohydrate fractions were in comparison with the results of other workers (Graham et al, 1986; Guisi-Perier et al, 1989; Stanogias and Pearce, 1985).

The results of the digestibility experiments indicate that with the inclusion of a high level of grain byproducts, oilseedmeals and pulps a greater proportion of the dNFE fraction is fermented. The energetic value of these feeds might be overestimated by the Rostock formula because, as mentioned, the energetic value of post-ileal fermented NFE is lower than of praecaecal digested NFE. Another important factor that might contribute to the inaccuracy of the estimation of the energy content of diets with a high NFE minus starch and crude fibre content is that the net energy from the fat digestion is overestimated in these diets (Jongbloed, 1986).
More research has to be done to understand the influence of the carbohydrate fraction from various raw materials on the digestion, and for the precise formulation of an adapted formula for the estimation of the energy content of feedstuffs.

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List of contributors to the scientific program

AUSTRALIA

P.D. Cranwell
D.B. Hennessy
R.J. Xu
School of Agriculture
La Trobe University
Bundoora Victoria 3083
AUSTRALIA

BELGIUM

P. Leterme
L. Pirard
A. Thewis
Faculté des Sciences Agronomiques de l'Etat
Unité de Zootechnie
Passage des Déportés 2
B-5800 Gembloux
BELGIUM

M. van Belle
Lab. de Biochemie de la Nutrition
Fac. des Sciences Agronomique
1348 Louvain-la-Neuve
BELGIUM

E. François
Ministère de l'Agriculture
Station de Chimie et Physique agricoles
B-5030 Gembloux
BELGIUM

BRASIL

C. Bellaver
E.T. Fialho
Department of Swine Nutrition
Nat. Research Center of Swine and Poultry
EMBRAPA/CNPSA
Concordia, SC.
BRASIL

CANADA

C.F.M. de Lange
W.C. Sauer
University of Alberta
Dept. of Animal Science
Faculty of Agriculture and Forestry
Edmonton
CANADA T6G 2P5

I. Galibois
H. Malouin
L. Savoie
Département de Nutrition Humaine
et de Consommation
Université Laval
Québec
CANADA

M.R. Bedford
H.L. Classen
J.F. Patience
Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon
Saskatchewan
CANADA S7K O17

DENMARK

P.T. Sangild
Dept. of Animal Sci. and Animal Health
Royal Vet. and Agric. University
Rolighedsvej 23
1958 Frederiksberg C.
DENMARK

K.E. Bach Knudsen
S. Boisen
B.O. Eggum
J.A. Fernàndez
B.B. Jensen
A. Just
National Institute of Animal Science
Dept. Animal Physiology and Biochemistry
Forsøgsanlaeg Foulum
Postboks 39, DK-8830 Tjele
DENMARK

B. Foltmann
Institute of Biochemical Genetics
University of Copenhagen
øster, Farimagsgadt 2A
1353 Copenhagen K
DENMARK

K. Mortensen
H. Sørensen
Chemistry Department
Royal Veterinary and Agricultural University
Thorvaldsensvej 40
DK-1875 Frederiksberg C
DENMARK

O. Noren
H. Sjöström
L. Wetteberg
Department of Biochemistry C
The Parrum Institute
University of Copenhagen
Blegdamsvej 3c
DK-2200 Copenhagen N
DENMARK

FINLAND

M. Näsi
H. Siljander-Rasi
University of Helsinki
Department of Animal Husbandry
Viiki 21, SF-00710 Helsinki
FINLAND

FRANCE

T. Corring
A. Rerat
I.N.R.A.
Lab. de Physiologie de la Nutrition
Domaine de Vilvert
78350 Jouy-en-Josas
FRANCE

J.C. Cuber
C. Juste
A. Langlois
C. Philippe
P. Valette
I.N.R.A.
Lab. d'Ecologie et de Physiologie du Systeme Digestif
Domaine de Vilvert
78350 Jouy-en-Josas
FRANCE

L. Buéno
J. Fiaramonti
V. Theodorou
Station de Pharmacologie Toxicologie
180 Chemin de Tournefeuille
B.P. 3
31931 Toulouse
FRANCE

C. Simoes Nunes
Dept. NASA
INRA-CRJ
78352 Jouy-en-Josas
Cedex
FRANCE

A. Aumaitre
J. Bengala Freire
E. Chabeauti
C. Fevrier
Y. Jaquelin
Y. Lebreton
J. Peiniau
Station de Recherches sur l'Elevage des Porcs
35590 Saint-Gilles, L'Hermitage
FRANCE

M.P. Le Guen
Euretec Gie
12 Avenue George V
75008 Paris
FRANCE

J.A. Chayvialle
Inseron
Hôpital E. Herriot
Lyon
FRANCE

B. Carré
INRA-SRA
Nouzilly
37380 Monnaie
FRANCE

GERMANY

F. Ahrens
M. Schmitz
J. Schön
IS Forschungsgesellschaft für Experimentelle Tierphysiologie und Tierernährung mbH and Co. KG
Wiesenweg 10a
Postfach 1247
2362 Wahlstedt
GERMANY

H. Henkel
R. Mosenthin
A. Pfeiffer
Institute of Animal Nutrition
University of Kiel
Olshausenstrasse 40-60
D2300 Kiel - 1
GERMANY

E. Borgmann
T. Heinz
U. Hennig
F. Kreienbring
U. Pöhland
W.B. Souffrant
D. Thomaneck
T. Völker
J. Wünsche
Forschungszentrum für Tierproduktion
Bereich Tierernährung "Oskar Kellner"
Justus von Liebigweg PF 27-01/02
Rostock 2500
GERMANY

H. Bergner
Sektion Tierproduktion und Veterinär Medizin, bereich Tierernährung
Humbold Universität
Invalidenstrasse 42
Berlin 1040
GERMANY

G. Breves
Institut für Vet. Physiologie
Justus von Liebig Universität
Frankfurterstrasse 100
D-6300 Giessen
GERMANY

W. Drochner
Institut für Tierernährung
Tierärztliche Hochschule
Bischofsholer Damm 15
D-3000 Hannover 1
GERMANY

G. Gebhardt
R. Köhler
F. Liebert
F. Reinisch
C. Wecke
Karl Marx Universität
Wissenschaftsbereich Tierernährungsphys. und Futtermittelkunde
Gustav Köhnstrasse 8
7022 Leipzig
GERMANY

J. Dreyer
Institute of Animal Nutrition
Federal Agricultural Research Centre
Braunschweig
GERMANY

H. Hagemeister
Institut für Physiologie und Biochemie der Ernährung
Bundesanstalt für Milchforschung
Kiel
GERMANY

HUNGARY

Z. Henics
J. Tossenberger
Division of Animal Nutrition and Feed Science
Faculty of Animal Production
Kaposvár
HUNGARY

JAPAN

S. Kaji
Shu Furuya
Department of Animal Production
Tohoku Nat. Agric. Experiment Station
Morioko 020-01
JAPAN

THE NETHERLANDS

H. Boer
B. Kemp
T. Köhler
C.A. Makkink
A.F.B. van der Poel
M.W.A. Verstegen
E.R. Wahle
Agricultural University Wageningen
Dept. of Animal Nutrition
Haagsteeg 4
6708 PM Wageningen
THE NETHERLANDS

M.J. van Baak
G.M. Beelen
G.B. Derksen
J.M. Fentener van Vlissingen
J. Huisman
G.J.M. van Kempen
P. van Leeuwen
E.C. Rietveld
J.B. Schutte
G.H. Tolman
E.J. van Weerden
J. Wiebenga
TNO-ILOB
Postbus 15
6700 AA Wageningen
THE NETHERLANDS

J.G.M. Bakker
H. Everts
P.W. Goedhart
R. Hoste
L.P. Jager
A.W. Jongbloed
P.A. Kemme
E. Krol-Kramer
N.P. Lenis
J. van der Meulen
Z. Mroz
H. de Visser
A.M. van Vuuren
IVVO
Postbus 160
8200 AD Lelystad
THE NETHERLANDS

H.J. van Lonkhuysen
Biochemical and Physical Chemistry Dept.
TNO Biotechnology and Chemistry Inst.
P.O. Box 360
3700 AJ ZEIST
THE NETHERLANDS

G.J. Borggreve
J.J. Heeres-Van der Tol
C.H.M. Smits
W.A.G. Veen
CLO "De Schothorst"
Postbus 533
8200 AM Lelystad
THE NETHERLANDS

A.C. Beynen
C.E. West
Dept. of Human Nutrition
Agricultural University Wageningen
Bomenweg 2
6703 HD Wageningen
THE NETHERLANDS

L.A. den Hartog
Research Institute for Pig Husbandry
P.O. Box 83
5240 AB Rosmalen
THE NETHERLANDS

D.J. van Zuilichem
Dept. of Food Technology,
section Process Engeneering
Bomenweg 2
6703 HD Wageningen
THE NETHERLANDS

J.E. van Dijk
Central Veterinary Institute
P.O. Box 1076
8200 BB Lelystad
THE NETHERLANDS

F.G.M. Russell
Department of Pharmacology
Faculty of Medicine and Densitry
University of Nijmegen
THE NETHERLANDS

POLAND

L. Buraczewska
S. Buraczewski
J. Gdala
W. Grala
G. Janowska
Institute of Animal Physiology and Nutrition
Polish Academy of Sciences
05-110 Jablonna
POLAND

SWEDEN

G.M. Ekström
B.W. Karlsson
J.B. Karlsson
N. Pantzar
S.G. Pierzynowski
J. Svendsen
B.R. Weström
University of Lund
Dept. of Zoophysiology
Helgonavägen 3B
S-223 62 Lund
SWEDEN

P. Aman
H. Graham
J. Inborr
Swedish University
of Agricultural Sciences
S-750 07 Uppsala
SWEDEN

W. Lowgren
Swedish University of Agric. Science
Box 7051, S-750 07 Uppsala
SWEDEN

UNITED KINGDOM

L.A. Cadenhead
M.F. Fuller
Rowett Research Institute
Greenburn Road
Bucksburn
Aberdeen, AB2 9SB
UNITED KINGDOM

C.L. Jones
J.W. Sissons
AFRC Institute for Grassland
and Animal Production
Shinfield
Reading RG2 9AQ
UNITED KINGDOM

USA

J.T. Yen
USDA, ARS, NPA
Roman L. Hruska
US Meat Animal Research Center
Box 166
Clay Center, NE 68933
U.S.A.

M.R.M. Ballard
M. Harrison
Heartland Lysine, Inc.
8430 West Bryn Mawr Ave.
Suite 650
Chicago, IL 60631
U.S.A.

C.A. Cook
R.A. Easter
A. Gonzalez
B.J. Kerr
University of Illinois
at Urbana-Champaign
Department of Animal Sciences
Mumford Hall
1301 West Gregory Drive
Urbana, IL 61801
U.S.A.

T.R. Cline
D.M. Forsyth
D.T. Kelley
A.G. Mathew
J.A. Patterson
A.B. Scheidt
A.L. Sutton
Purdue University
Department of Animal Science
Poultry Science Building
West Lafayette, IN 47907
U.S.A.