NITROGEN UTILIZATION IN PIGS AS AFFECTED BY DIETARY INDUCED LOSSES OF ILEAL ENDOGENOUS NITROGEN

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PREFACE

This thesis is a result of a collaboration between the TNO-Nutrition and Food Research Institute, Department for Animal Nutrition and Physiology (ILOB) in Wageningen, Wageningen Agricultural University, Animal Nutrition Group (VV), the Netherlands, and Polish Academy of Sciences, The Kielanowski Institute of Animal Physiology and Nutrition, Jablonna, Poland.

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Grala, W. 1998. Nitrogen Utilization In Pigs As Affected By Dietary Induced Losses Of Ileal Endogenous Nitrogen. The results of this thesis showed that various protein sources (feedstuffs) have different values for apparent and true ileal digestibilities of protein and thus also of ileal endogenous nitrogen losses (ENL; determined with the ^{15}N-isotope dilution technique). It was initially hypothesized that increased ENL may induce an increased recycling of endogenous proteins. As a result of the latter, increased nitrogen (N) losses may occur in urine because of inefficiency of metabolic processes. Furthermore, the increased ENL may lead to a decreased utilization of small intestinal dietary N for retention in pigs. In this context, when using feedstuffs that led to a distinctly different ENL, the results of N balance in pigs were in agreement with the starting hypothesis. Further, it was found that feedstuffs with protease inhibitor-related ENL reduced the dietary N utilization and increased urinary N loss. For feedstuffs with various ENL related to fibre *per se*, the urinary N loss and dietary N utilization for retention did not differ significantly. It means that ENL and N metabolism differ depending on the feed factors to which the ENL are related. These results indicated that (1) total secretion of endogenous N may not be related to the level of ENL, and (2) the reabsorption ratio of the secreted endogenous N may vary for different feedstuffs. Based on that, the starting hypothesis was modified to: mainly, the magnitude of ENL (i.e., first-limiting amino acids) determines the inefficiency of dietary N utilization rather than the magnitude of total recycled endogenous N. Thus, increased urinary N losses may depend on the extent of amino acid oxidation resulting from the amino acid imbalance caused by the ENL. In the particular experimental conditions (restricted protein/AA intake), extra supplementation with crystalline amino acids of a diet with high protease inhibitor-related ENL, improved the utilization of the dietary N and decreased extra urinary N loss. It seems, therefore, that extra amino acid addition can compensate for the ENL by improving the profile of amino acids available for endogenous protein re-synthesis. As a result, the remaining dietary amino acids are in a better balance for the body protein retention. Further results showed that the total amounts of endogenous N secretions were approximately similar for different dietary factors that induce the ENL. Therefore, the magnitude of the ENL rather than the magnitude of total secreted and reabsorbed endogenous N mainly determines the efficiency of dietary N utilization and extra urinary N loss. These results were in agreement with the adjusted hypothesis. Supplementation with extra amino acids may decrease environmental N losses, caused by increased ENL, and increase the efficiency of dietary N utilization in pig production.

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CONTENTS

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

It is increasingly recognised that intensive animal production is becoming harmful to the environment because of a low utilization of ingested nitrogen (N) for animal protein. Usually, more than 60% of the feed N (input) is excreted with faeces (15-20% of intake) and urine (40-50% of intake) in pig production (Jongbloed and Lenis, 1992). The amount of excreted N (output) in some places is so large that the N-cycling processes in nature can no longer handle this N excess. Thus, harmful N-containing compounds (ammonia, nitrous oxide, nitrogen oxide, nitrogen dioxide, nitrate) escape to the atmosphere and leak into the soil and/or ground water (Tamminga and Verstegen, 1991). Therefore, N losses must be reduced by improving the output to input ratio of N in animal production to maintain a high productivity without reducing the production volume.

Current research has already shown that there are some possibilities to reduce excessive N excretion by animals, using dietary manipulations such as:

- minimizing dietary N input by the use of synthetic amino acids (Schutte et al., 1993);
- increasing N digestibility of feeds and feedstuffs by decreasing the effects of anti-nutritional factors (ANF), using technological processing, such as heat treatment (eg., pelleting, extrusion, toasting) and dehulling (review of Melcion and Van der Poel, 1993);
- decreasing the effects of ANF and non-starch polysaccharides (NSP) by enzyme treatments (Classen et al., 1993; Cos et al., 1993).

A new area of research is a possibility to reduce N excretion by a reduction of the recycling and losses of endogenous N in pigs. According to Huisman et al. (1993), lowering N losses associated with the excretion of endogenous N could lead to reduced excretion of both urinary and faecal N in pig production. Until now, it was insufficiently recognised that a considerable part of the endogenous N may be excreted via the urine.

Generally, N losses are associated with incomplete digestion of feed protein and inefficient utilization of absorbed amino acids for protein synthesis and production, because of their imbalance and/or use for other metabolic purposes (e.g., maintenance processes, energy source).

Apparently, undigested protein in the ileal digesta and faeces comprises both feed protein and non-absorbed endogenous secretions. Important sources of endogenous nitrogenous compounds in the gastro-intestinal tract are saliva, gastric juice, pancreatic juice, bile, intestinal juices, mucosa and sloughed mucosal cells (Souffrant, 1991). True protein digestibility of most common feedstuffs used in pig nutrition is often above 90% while apparent digestibility is distinctly lower (about 15-20 digestibility units) (Huisman et al., 1993). Thus, the difference between apparent and true digestibility indicates that most feed proteins are highly hydrolysable by the digestive enzymes. It appears, therefore, that a lower apparent protein digestibility does not necessarily result from a lower true protein digestibility, but may often be caused by a higher level of endogenous N in chyme and/or faeces.

Various methods have been used to distinguish between endogenous protein and feed (exogenous) protein in the gastro-intestinal tract. The methodology, limitations and difficulties of each of the methods, listed below, have recently been reviewed in detail by Souffrant (1991), Tamminga et al. (1995), Boisen and Moughan (1996a), and Nyachoti et al. (1997). In earlier studies, conventional methods were used for the estimation of endogenous gut N loss(es) (ENL). These methods are feeding protein-free diets, feeding diets containing protein sources (assumed to be 100% digestible), and mathematical regression methods with the use of graded protein levels in diets and extrapolated output of protein (N) to a protein-free diet. One interesting method is the mobile nylon bag technique (MNB), which has got a great potential for the determination not only the true digestibility of feed protein (N), but also digestibility of individual amino acids of feed protein and the amino acid pattern of endogenous protein.

At present, several other alternative techniques are used to estimate the ENL in pigs fed protein-containing diets. These methods are the homo-arginine technique, isotope dilution techniques with the use of ^{15}N to label either the animal or dietary N, and enzymatically hydrolysed casein technique (EHC), also known as the peptide alimentation ultrafiltration method. Results obtained with the latter two methods indicate that estimations of the ENL are generally higher and give grater differences between diets than those with the earlier methods. The ^{15}N-isotope dilution technique is the most commonly used method to estimate differences in the ENL in pigs depending on various dietary factors.

The level of endogenous N in ileal digesta of the pig is influenced by animal factors, such as age and body weight (Mariscal-Landin et al., 1995), and by a number of dietary factors. The most important dietary factors are dry matter intake (Butts et al., 1993b), level and quality of feed protein (De Lange et al., 1990; Butts et al., 1993a), presence of anti-nutritional factors (ANF), such as trypsin inhibitors (Barth et al., 1993; Schulze, 1994), lectins (Schulze, 1994), and tannins (Jansman et al., 1995). The extent of ENL is also associated with dietary carbohydrates (fibre). Fibre, mainly NSP, can be divided more specifically into cell wall components (NDF), ß-glucans and pectic substances (Mosenthin et al., 1994; Schulze et al., 1994, 1995; Leterme et al., 1996). Microflora, present in the gastro-intestinal tract, may incorporate N in their protein and may, therefore, impair the determination of the digestibilities of feed protein (De Lange et al, 1992). Endogenous protein can be degraded by bacteria in the distal small intestine (Boisen et al., 1985). It may also be metabolized and incorporated into bacterial protein (De Lange et al., 1992; Schulze et al., 1994).

The ENL at the terminal ileum of pigs can be divided into a basal loss and extra (specific) loss. The basal endogenous loss is related to the dry matter intake of the non-specific components in the diet. In that respect, the animal factors may interfere with the basal endogenous protein loss. The basal losses of endogenous protein (N x 6.25) range mostly from about 10 to 16 g/kg of dry matter intake, depending on the investigation and method used (Wünsche et al., 1987; De Lange et al., 1989; Leterme et al., 1992, 1996; Fan et al., 1995; P. van Leeuwen, personal communication).

The extra endogenous loss is related to any specific dietary factors of the protein sources. In practice, the specific losses may lead to the differences in the ENL between pigs fed various feedstuffs. These differences are proportional to the amounts of the specific feedstuff-related factors (Schulze, 1994). The specific losses may considerably exceed the basal losses, in extreme cases even being up to 6-7-fold greater (Tamminga et al., 1995; Boisen and Moughan, 1996a, 1996b; Nyachoti et al., 1997).

Sufficient energy supply (Bikker, 1994) and an optimal pattern of most essential amino acids (Fuller et al., 1989; Wang and Fuller, 1989) is needed for a maximum utilization of amino acids for protein deposition rather than the total protein (Lenis, 1992; Schutte et al., 1993). In that respect, both endogenous and exogenous amino acids, absorbed before the end of the small intestine, may only be of nutritional importance (Żebrowska et al., 1978). The undigested protein is passed to the large intestine and is not utilized for protein synthesis. This protein is excreted mainly as bacterial N with faeces and/or is absorbed as ammonia, converted in urea or uric acid and contributes to urinary N loss (Low and Żebrowska, 1989).

The dietary supply of amino acids for metabolism depends not only on ileal digestibilities, but also on their post-absorptive availabilities (Hurrell, 1990; Batterham, 1992). The latter may be negatively influenced by severe heat treatments, as was shown for lysine (Anderson-Hafermann et al., 1993; Grala et al., 1994; Van Barneveld et al., 1994). Batterham (1992) suggested that availability of amino acids of heat-treated feedstuffs falls more than does the apparent ileal digestibility. Additionally, amino acids may vary in their sensitivity to processing conditions (e.g., Lys, Thr, Met, and Trp) and the ileal digestibility may overestimate availability of such amino acids for protein synthesis. If these amino acids are rendered unavailable for protein metabolism, they will be excreted in urine (Finot, 1990). As a consequence, other amino acids are in excess. The same holds true when pigs are fed readily available, but unbalanced dietary amino acids. Similar loss of amino acids may occur when pigs will be fed with feeds that induce significant specific ileal losses of endogenous essential amino acids. For instance, vast losses of methionine and cystine can be expected when feeding ingredients with a high content of trypsin inhibitors (Liener and Kakade, 1980). While feeding feeds with a specific NSP, extra threonine losses can occur (Mosenthin et al., 1994). These specific losses of amino acids may cause an imbalance in the profile of recycled endogenous amino acids. Recycling of endogenous protein involves secretion, re-digestion/reabsorption and resynthesis.

During metabolic processes there is no mechanism to store excessive amino acids, so only amino acids that are balanced will be used preferentially for protein synthesis. The unbalanced amino acids will be catabolized by the amino-acid-degrading enzymes (Simon, 1989). The N-containing end-products (e.g. uric acid, urea, creatinine) are excreted in urine and contribute to N losses (Tamminga and Verstegen, 1991). Amino acids may also become an energy source for the metabolic processes in case of energy deficiency from other sources (Simon, 1989). Moreover, metabolic processes are very complex and allow N (amino acids) to circulate between various body compartments of the pig. Due to this amino acid reutilization and some inefficiency of the involved processes (Simon, 1989), some of the N losses, resulting from incomplete digestion of endogenous proteins, may not be excreted in the faeces, but in the urine (Huisman et al., 1993). The latter authors suggested that there may be a positive relationship between the magnitude of ENL and the extent of recycled endogenous N. Therefore, greater ENL may cause greater urinary N losses, resulting from the enhanced N losses during metabolic processes of the recycled endogenous N. Huisman et al. (1993) showed that about one-third of total urinary N losses may originate from endogenous N sources.

For the research in this thesis, it was assumed that greater ENL are associated with greater recycling of endogenous N. The greater demands for recycled amino acids will provoke greater N losses during metabolic processes. These N losses will occur due to amino acid degradation because of a basic inefficiency of the metabolic processes. As a result, the N-containing metabolites will contribute to extra urinary N losses. Therefore, it is hypothesized that increased ENL may not only lead to extra N in chyme and or faeces, but also in extra N losses in the urine. Moreover, as a consequence of the enhanced ENL, the efficiency of the dietary N utilization may be decreased in the pig.

Using several experimental designs (Chapters 1, 2, 4, and 5), a variety of protein sources was tested to identify their apparent and true ileal digestibilities of protein, and ENL in growing pigs. The effects of selected dietary factors (trypsin inhibitors, fibre/NDF) and/or technological processing (dehulling, toasting) on the above mentioned characteristics were also studied (Chapter 2). A combination of two experimental techniques was used: 1) PVTC-cannulation of pigs (Van Leeuwen et al., 1991) to determine apparent ileal digestibilities of protein and amino acids, and 2) the ^{15}N-isotope dilution technique (Schulze, 1994) to determine ENL and true ileal N digestibility.

In separate experimental designs (N balance trials) the partitioning of the ingested feed N between body, faecal excretion and urinary losses was determined in pigs. In Chapter 1, the validation of the hypothesis was studied; the effects of different levels of ENL on the N utilization and urinary N losses were studied. In Chapter 3, the validation of the hypothesis was tested in growing pigs when feeding feedstuffs with protease inhibitor- or fibre-dependent ENL and the ENL dependent on technological treatments (dehulling and toasting). The results of Chapter 3 led to a modification of the original hypothesis, so the modified hypothesis is evaluated in Chapter 5. The possibility to compensate for the negative effect of ENL on the N utilization and urinary N losses was studied, using a diet with high ENL and extra supplemented with crystalline amino acids. The effects of different protein sources (generally, high/low trypsin inhibitor or high/low NDF contents) on the small intestinal flow of endogenous and exogenous N and on the net reabsorption of endogenous N up to the terminal ileum was investigated in Chapter 6. Finally, the major findings of Chapters 1 through 6 are discussed in the General Discussion with respect to the possible mechanisms of endogenous protein reutilization and ENL, and their negative impact on the efficiency for dietary protein utilization for protein deposition.

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CHAPTER 1

NITROGEN BALANCE OF PIGS AS AFFECTED BY FEEDSTUFFS CAUSING DIFFERENT ENDOGENOUS NITROGEN FLOW AT THE TERMINAL ILEUM

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Nitrogen balance of pigs as affected by feedstuffs causing different endogenous nitrogen flow at the terminal ileum

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Abstract

The effect of ileal endogenous nitrogen (N) flow on N balance in pigs was investigated. In two experiments pigs (BW 12 to 24 kg) were fed a maize starch-based diets containing either wheat gluten (WG), peas, or rapeseed meal (RSM). In Experiment 1, apparent ileal N digestibility was measured with 12 ileal cannulated pigs. Amount of ileal endogenous N was measured in six of these pigs with the ^{15}N-isotope dilution technique. Apparent and true ileal N digestibilities were 91.4 and 98.6 for the WG diet, 80.5 and 95.6 for the pea diet, 67.2 and 80.1% for the RSM diet, respectively. Significant differences ($P < 0.05$) were observed in ileal flows of dietary N, endogenous N, and DM. In Experiment 2, thirty pigs were used to study N balance. Diets were balanced for the contents of apparent ileal digestible (ID) CP (10.8%) and ID essential amino acids. Faecal N digestibility decreased from 92.9 to 82.1 and 72.4% in the WG, pea and RSM diets, respectively, corresponding to increased urinary N excretion and decreased N retention. The efficiencies of ID CP utilization were 83.5, 79.7 and 75.0%, respectively. The study showed that pigs fed diets with an increased flow of endogenous ileal N concomitant increase in excretion of urinary N. Nitrogen retention and utilization of ileal digestible N in those pigs were also decreased.

Key words: Pigs; Endogenous Nitrogen; Digestibility; Nitrogen Balance; ^{15}N-Dilution Technique

Introduction

Amino acids that are not absorbed before the end of the ileum are of little value as precursors for protein synthesis in the pig, because there is negligible amino acid absorption in the large intestine (Żebrowska et al., 1978). This applies to both dietary and endogenous amino acids. Huisman et al. (1993) demonstrated that a low apparent ileal digestibility of dietary protein in pigs is often caused by a high excretion of endogenous N at the terminal ileum. From Souffrant (1991) and Krawielitzki et al. (1994) it can be derived that about 25% of secreted endogenous N in the digestive tract is not reabsorbed before the terminal ileum. So, the total amount of endogenous protein secreted into the lumen of the intestinal tract may be four times greater than that recovered at the terminal ileum. The increased secretion of endogenous N (amino acids) will be associated with an increased (re)synthesis of endogenous protein. Absorption and metabolism coincide with some inefficiency. Only part of (re)absorbed endogenous amino acids is added to the total pool of amino acids. They can be used for protein (re)synthesis and other purposes. This means that some losses occur because of imbalance in the profile of amino acids and also due to losses during protein

Table 1. Chemical composition (g/kg, as-fed basis) of wheat gluten, peas and rapeseed meal used in Experiment 1 and Experiment 2

Item	Wheat gluten	Peas	Rapeseed meal
Proximate analysis			
Dry matter	916.2	897.2	916.0
Crude protein (N x 6.25)	817.8	212.8	316.0
Ether extract	43.5	19.5	32.5
Crude fibre	7.0	64.3	112.3
NDF[1]	10.5	169.3	253.5
Ash	7.9	33.2	71.8
TIA[2] (mg/kg of product)	ND[3]	28.4	ND
Indispensable amino acids			
Arginine	29.0	19.8	16.8
Histidine	17.4	4.8	9.1
Isoleucine	30.7	9.0	13.3
Leucine	60.2	15.1	23.8
Lysine	13.7	14.1	17.4
Methionine	12.7	2.1	6.4
Phenylalanine	43.8	9.9	13.9
Threonine	21.5	8.1	14.8
Tryptophan	6.8	1.8	4.5
Valine	33.0	10.4	16.9
Dispensable amino acids			
Alanine	21.7	9.3	15.0
Aspartic acid	26.9	22.0	25.2
Cystine	16.4	3.1	6.8
Glutamic acid	346.4	35.3	58.3
Glycine	27.6	9.0	17.2
Proline	102.4	8.9	20.2
Serine	40.7	10.6	14.6
Tyrosine	27.5	7.2	10.6

[1] Neutral detergent fibre.

[2] Trypsin inhibitor activity.

[3] Not determined.

synthesis. The resulting N will appear in urine (Moughan, 1993). Huisman et al. (1993) calculated that about 23% of urinary N (in relation to N intake) may originate from the intermediary metabolism of endogenous protein. If the flow of endogenous N at the pig's terminal ileum is only 25% of the total secretion, it can be assumed that a higher flow of endogenous ileal N will lead to a higher urinary N loss. This may negatively affect N balance in the pig.

The present investigation was undertaken to determine the flow of endogenous N at the terminal ileum consequent upon giving three different protein sources to growing pigs, and to determine N balance in pigs fed well balanced diets containing these protein sources.

Materials and methods

Feedstuffs

Three protein-rich feedstuffs were used in two experiments. Wheat gluten (WG) was chosen as a highly digestible source of protein inducing a relatively low flow of endogenous N at the terminal ileum. Peas (*Pisum sativum*) were used as a feedstuff with a high true ileal N digestibility, but inducing a high flow of ileal endogenous N (Huisman et al., 1992). Rapeseed (*Brassica napus*) meal with a low content of glucosinolates (RSM) was used for both its low true and apparent ileal N digestibilities, and a high flow of ileal endogenous N. The chemical characteristics of these feedstuffs are presented in Table 1.

Determination of apparent ileal N digestibility and measurement of the ileal flow of endogenous N (Experiment 1)

Animals and housing

The experiment was performed using 12 crossbred barrows [(Dutch Landrace x Yorkshire) x Finnish Landrace]. At the start of the experiment, piglets were about five to six week old and had a mean BW (± SE) of 9.9 kg (± 0.22 kg). Barrows were individually housed in metabolism cages (80 x 180 cm) at an ambient temperature of 22 to 24 °C and a relative air humidity of about 55%. They could move freely. Barrows were randomly assigned to three dietary treatments (Table 2). The experiment was reviewed and approved by the TNO Committee for Animal Welfare.

Table 2. Ingredient composition of the experimental diets (as-fed basis)

	Experiment 1[1]			Experiment 2		
Diet:	Wheat gluten	Pea	RSM	Wheat gluten	Pea	RSM
Ingredient (g/kg)						
Wheat gluten	178.0	-	-	123.50	-	-
Peas	-	755.0	-	-	597.0	-
Rapeseed meal	-	-	508.0	-	-	500.00
Maize starch	518.3	15.0	237.5	569.85	170.4	244.66
Dextrose	150.0	150.0	150.0	150.00	150.0	150.00
Soya oil	20.0	30.0	60.0	11.00	20.0	58.00
Cellulose	50.0	-	-	50.00	-	-
Vitamin/mineral mixture[2]	10.0	10.0	10.0	10.00	10.0	10.00
$CaCO_3$	10.0	12.0	5.0	10.00	12.0	5.00
$CaHPO.H_2O$	25.0	18.0	17.0	25.00	19.5	17.00
NaCl	5.0	5.0	5.0	5.00	5.0	5.00
MgO	2.0	-	-	2.00	1.0	-
$KHCO_3$	20.0	-	3.0	20.00	2.0	3.00
$NaHCO_3$	5.0	3.0	3.0	5.00	3.0	3.00
L-Lysine.HCl	6.2	-	1.5	8.50	0.8	3.00
DL-Methionine	-	1.5	-	1.10	1.8	-
L-Threonine	0.5	-	-	2.70	1.7	0.80
L-Tryptophan	-	0.5	-	0.85	1.0	0.44
L-Isoleucine	-	-	-	0.90	0.5	0.10
L-Cystine	-	-	-	0.70	1.7	-
L-Valine	-	-	-	1.70	1.2	-
L-Histidine	-	-	-	0.80	0.1	-
L-Leucine	-	-	-	1.40	1.3	-

[1] To each diet in Experiment 1 Cr_2O_3 was added (2.5 g/kg) as a digestibility marker.

[2] The vitamin/mineral mixture supplied per kg feed: 9000 IU vit. A; 1800 IU vit. D_3; 40 mg vit. E; 5 mg riboflavin; 30 mg niacin; 12 mg pantothenic acid; 100 mg choline, 40 µg vit. B_{12}; 2 mg vit. B_1; 3 mg vit. B_6; 0.1 mg biotin; 1 mg folic acid; 3 mg vit. K; 50 mg ascorbic acid; 300 mg $ZnSO_4.H_2O$; 15 mg MnO_2; 400 mg $FeSO_4.7H_2O$; 2.5 mg $CoSO_4.5H_2O$; 0.5 mg KI; 35 mg $CuSO_4.5H_2O$; 0.2 mg $Na_2SeO_3.5H_2O$; 40 mg Tylosin.

Diets and feeding

Wheat gluten, peas and RSM were the only protein sources in the respective maize starch-based diets (Tables 2 and 3). The diets were formulated to contain equal amounts of CP and net energy (NE). Contents of apparent ileal digestible lysine, threonine, tryptophan, or sum of methionine and cystine corresponded at least to the required levels for pigs of 10 to 25 kg BW (CVB, 1992). The diets were supplemented with marginal amounts of synthetic amino acids to accomplish a minimum level for the limiting essential amino acids (CVB, 1992) to ensure an optimum pigs' growth. A factor of 0.7 was used to convert metabolizable energy (ME) to NE. Chromic oxide was added to the diets as a marker (2.5 g/kg as-fed diet). During the experimental period, the diets were fed twice a day in equal meals at 0800 and 2000 h. Pigs were fed individually at the level of 2.6 times the ME requirement for maintenance (ARC, 1981). Feed was mixed with water (1:2, wt/vol) just before feeding. No additional water was supplied.

Experimental procedure

The scheme of the experiment is presented in Figure 1. After an adaptation period (13 d), twelve pigs were surgically fitted with a Post-Valve-T-Caecum cannula (Van Leeuwen et al., 1991). The recovery period (eight to nine days) was followed by the insertion of two catheters into blood vessels of six of the 12 cannulated pigs. The first catheter was placed into the external jugular vein (for blood sampling), and the second into the carotid artery (for the infusion of $[^{15}N]$-Leucine). During a preliminary period (adaptation), pigs were fed a commercial diet. During the recovery period, they were offered a limited amount of feed and the experimental diets were gradually introduced.

	Preliminary period						Experimental period												
Day	1 - 13	14	15	15 - 23	24	25	1 - 6	7	8	9	10 - 15	16	17	18	19 - 24	25	26	27	28
	Adaptation	PVTC[1]		Recovery	Catheters[2]			1st digesta collection				2nd digesta collection				3rd digesta collection			F[3]
							$[^{15}N]$-Leucine infusion												

Figure 1. Scheme of the experimental procedure.

[1] Ileal cannulation using Post-Valve-T-Caecum cannula (Van Leeuwen et al., 1991).

[2] Insertion of two blood catheters into blood vessels; the first one placed into the external jugular vein (for blood sampling), and the second into the carotid artery (for the infusion of $[^{15}N]$-Leucine).

[3] End of the experiment.

The twelve pigs were used in a crossover design originally comprising five treatments and three periods. Two of these treatments are not reported in this publication. Each of the periods consisted of one day for exchanging diets plus five adaptation days to the diets (total six days), and of three successive days of continuous digesta collection from 0800 to 2000 h. The first period started one week after the beginning of [^{15}N]-Leucine infusion in the six catheterized pigs. The next two periods followed at one week intervals.

A continuous 27-d infusion of [^{15}N]-Leucine in the catheterized pigs started one day after catheterization. The infusion rate was 5.04 mg of [^{15}N]-Leucine (95% ^{15}N-enrichment) kg^{-1} BW/d. The [^{15}N]-Leucine was dissolved in a sterile non-pyrogenic, physiological saline solution (NaCl, 9 g/L) and was infused by perfusion pumps (Fr. B. Braun Melsungen AG, Germany). The 'Swivel technique' was applied to infuse continuously freely moving pigs (Van Kleef, 1993). Blood samples (about 8 ml) were taken once daily from the infused pigs during the digesta collections at 1400 h each day. After sampling, blood samples were treated as described by Schulze et al. (1995).

The apparent ileal digestibility coefficients for DM and N were determined by the chromic oxide ratio method. Since the contribution of N of synthetic amino acids to total dietary N was negligible, no adjustment was made for the synthetic amino acids added to the diets. The recovery of ileal endogenous N was calculated from the ratio of ^{15}N-enrichment excess in ileal digesta to that in the TCA-soluble blood plasma fraction, using the adapted formula of Souffrant et al. (1981) and de Lange et al. (1990):

$$N_e = N_d \times [(E_d - E_{nd})/(E_{pl} - E_{npl})], \qquad [1]$$

where N_e is the endogenous N flow (g/d and g/kg DMI); N_d is the total N in ileal digesta (g/d); E_d is the ^{15}N-enrichment in ileal digesta; E_{nd} is the natural background ^{15}N-enrichment in the diet; E_{pl} is the ^{15}N-enrichment in the TCA-soluble blood plasma; E_{npl} is the natural background ^{15}N-enrichment in the TCA-soluble blood plasma. Thus, (E_d - E_{nd}) stands for the ^{15}N-enrichment excess in ileal digesta, and (E_{pl} - E_{npl}) stands for the ^{15}N-enrichment excess in the TCA-soluble blood plasma.

To calculate the true ileal digestibility of N, the apparent ileal digestible N was corrected for the endogenous N in the digesta.

At the beginning of the experimental period the average BW of the 12 pigs was 13.7 kg (± 0.29 kg). At the end of experiment the average BW was 22.3 kg (± 0.39 kg).

Nitrogen balance trial (Experiment 2)

Animals and housing

The experiment was performed using 30 crossbred barrows [(Dutch Landrace x Yorkshire) x Finnish Landrace] that were seven to eight week old and had a mean BW of 15.2 kg (± 0.22 kg) at the start. Pigs were individually housed in metabolism cages at an ambient temperature of 22 to 24°C and a relative air humidity of about 55 percent. Barrows were randomly distributed among three dietary treatments (Tables 2 and 3).

Diets and feeding

The WG, peas and RSM were the only protein sources in the maize starch-based diets (Table 2). The diets were formulated to contain similar amounts (10.8%) of apparent ileal digestible CP. As a result, the total CP contents in the diets were different (Table 3). Digestibility values for apparent ileal digestible CP determined in Experiment 1 were used in the diet formulation. Amino acid contents in feedstuffs were determined (Table 1), and tabular values were obtained for the digestibility coefficients (DC) of lysine, sum of methionine and cystine, threonine, and tryptophan (CVB, 1992). The DC for isoleucine in WG, peas, and RSM were estimated according to Van Leeuwen et al. (1993). The DC of isoleucine in the three feedstuffs were 92, 76 and 71%, respectively.

The diets were supplemented with synthetic amino acids. Contents of apparent ileal digestible lysine, threonine, tryptophan, and isoleucine were fixed at 85% of requirement for pigs of 10 to 25 kg BW (CVB, 1992). Methionine was at 90%, and the sum of methionine and cystine was at 100% of the levels of the Dutch requirement values (CVB, 1992).

Pigs were fed three times a day in equal meals at 0800, 1300 and 1800 h at the level of 2.7 times the ME requirement for maintenance (ARC, 1981). Feed was mixed with water (1:2; wt/vol) just before feeding. No additional water was supplied after feeding.

Experimental procedure

Pigs were distributed among three 10-pig groups according to the stratified BW. After an adaptation period (nine days) on the commercial diet, experimental diets were introduced. After two weeks of adaptation to the experimental diets, N balance period started, which lasted seven days. The average BW of barrows at the beginning of the N balance period was 21.3 kg (± 0.24 kg), and at the end 24.1 kg (± 0.27 kg). Faeces were quantitatively collected in plastic bags attached to the pigs (Van Kleef et al., 1994).

Table 3. Chemical composition of the experimental diets (as-fed basis)

	Experiment 1[1]			Experiment 2[2]		
Diet:	Wheat gluten	Pea	RSM	Wheat gluten	Pea	RSM
Nutrients as analyzed (g/kg)						
Dry matter	911.9	885.9	917.0	903.2	903.1	912.3
CP (Nx6.25)[3]	160.1	161.1	157.1	119.9	136.9	159.8
Ether extract	22.1	40.4	71.4	8.5	21.0	41.5
Crude fibre	50.4	47.6	54.4	39.7	45.0	62.5
NDF[4]	ND[5]	ND	ND	54.0	106.0	131.0
Amino acids as estimated[6] (g/kg)						
Lysine	7.4	11.8	9.0	8.4	9.6	10.1
Methionine	2.6	3.1	3.3	2.8	3.0	3.3
Methionine+Cystine	6.0	5.3	7.3	5.7	6.5	7.2
Threonine	4.6	6.5	6.8	5.4	6.6	7.4
Isoleucine	6.4	7.3	6.6	5.1	6.0	6.6
Tryptophan	1.4	2.0	1.9	1.7	2.1	2.3
Apparent ileal digestible (ID)-CP and amino acids (g/kg)						
ID-CP				108.0	108.0	108.0
ID-Lysine				8.1	8.1	8.1
ID-Methionine				2.7	2.7	2.7
ID-Methionine+Cystine				5.5	5.5	5.5
ID-Threonine				5.1	5.1	5.1
ID-Isoleucine				4.7	4.7	4.7
ID-Tryptophan				1.7	1.7	1.7
NE (MJ/kg)[7]	9.9	9.9	9.9	9.9	9.9	9.9

[1] In Experiment 1, diets are balanced for NE, CP and for the apparent ileal digestible requirements of the five most limiting amino acids (CVB, 1992).

[2] In Experiment 2, ileal digestible amino acids in the diets were balanced at 85% of CVB (1992) for the apparent ileal digestible amino acids allowances in feeds for pigs (BW of 10-25 kg) with the exceptions of Methionine (90%) and Methionine+Cystine (100%). The determined values of the apparent ileal digestibility of CP in feedstuffs are presented in Table 4.

[3] Crude protein (Nx6.25).

[4] Neutral detergent fibre.

[5] Not determined.

[6] Amino acid contents were calculated from their analyzed contents in the ingredients.

[7] Net energy; based on the tabular NE values for the individual feed ingredients (CVB, 1992).

Bags were changed twice a day, frozen and kept at -20°C until faeces analyses. Urine was collected in plastic containers via funnels underneath the cages. Urine was removed once a day and stored at 4°C until analyses. To prevent N losses during collection and storage, 8 mL of 25% H_2SO_4 was added to each container every day.

The apparent faecal DC of N for the WG, pea and RSM diets were calculated, after correcting for N added to the diets as synthetic amino acids. Synthetic amino acids were assumed to be 100% absorbed before the end of ileum. Nitrogen intake (g/d), faecal and urinary N excretions (g/d) were measured during the N balance. Nitrogen absorbed (g/d) was calculated as N intake minus faecal N, and N retention (g/d) was calculated as N intake minus faecal N minus urinary N.

Chemical analyses

In Experiment 1, samples of ileal digesta were pooled per pig and diet within experimental period. In Experiment 2, samples of faeces and urine were pooled per pig for the entire seven day period of the N balance. In both experiments, N and DM in the diets, freeze-dried ileal digesta and faeces, and N in urine were analyzed according to standard methods (AOAC, 1984). Neutral detergent fibre (NDF) was determined as described by Englyst and Cummings (1988). Chromium in the diets and ileal digesta was determined according to Bosch et al. (1988). The content of trypsin inhibitors in peas was analyzed according to a modified method of Kakade (Van Oort et al., 1989). The ^{15}N-enrichment of ileal digesta, feed, and of the trichloroacetic acid (TCA)-soluble fraction of blood plasma was determined according to the procedure described by Schulze et al. (1995).

Statistical analysis

Statistical analysis of data in both experiments was performed using the GLM procedure of SAS (1990). In Experiment 1, since the crossover design was used, and two treatments of five were not reported, six observations on infused pigs and six observations on just cannulated pigs were treated as missing values. The effects of dietary treatment and period of ileal digesta collection on the studied traits were analyzed according to the following model:

$$Y_{ijkl} = \mu + T_i + A_j + P_k + e_{ijkl}, \qquad [2]$$

where: Y_{ijkl} = dependent variable, μ = overall mean, T_i = treatment as diet (i = 1, 2, 3), A_j = animal (j = 1, ..., 6 for measuring endogenous N flow using ^{15}N, and j = 1, ..., 12 for determination of the apparent ileal digestibility), P_k = period (k= 1, 2, 3), and e_{ijkl} = residual error.

The apparent ileal digestibilities of DM and N were calculated according to data of eight (the WG and RSM diets), or seven (pea diet) observations (12 cannulated pigs). The true ileal N digestibility was calculated according to data of four (the WG and RSM diets), or three (pea diet) observations on the six catheterized pigs. The ileal flows (g/d) of total N, dietary N and endogenous N were also calculated using four (the WG and RSM diets), or three (pea diet) observations. Additionally, the ileal flows (g/kg DMI) of endogenous N and DM were calculated using the corresponding observations.

In Experiment 2, the effect of dietary treatment (T) on the components of the N balance (apparent faecal digestibility of DM and N, N intake, faecal N, urinary N, and N retention) was tested against the residual error (e) according to following model:

$$Y_{ij} = \mu + T_i + e_{ij}, \qquad [3]$$

where: Y_{ij} = dependent variable, μ = overall mean, T_i = treatment as diet (i = 1, 2, 3), e_{ij} = residual error.

When a significant effect of a dietary treatment was found, differences among means were compared, using the Tukey's test (SAS, 1990).

Table 4. Apparent ileal digestibilities of dry matter and nitrogen in Post-Valve-T-Caecum cannulated pigs fed maize starch-based diets containing either wheat gluten, peas or rapeseed meal as the sole protein source (Experiment 1)

	Diet			RMSE[1]
	Wheat gluten	Peas	Rapeseed meal	
No. of observations	8	7	8	
Apparent ileal digestibility (%)				
Dry matter	89.4^a	77.6^b	72.8^c	1.12
Nitrogen	91.4^a	80.5^b	67.2^c	2.61

[1] Root mean square of the error term; the degrees of freedom of the error term are 20 (23 observations).

[a, b, c] Means within a row lacking a common superscript letter differ at $P < 0.05$.

Results

In both experiments, pigs remained healthy and no feed refusals were recorded. In Experiment 1 (period 3), due to technical problems with the ^{15}N-infusion, one pig was excluded from the trial (pea treatment). The statistical analysis, using model [2] showed that effects of period (P) and animal (A) were not significant ($P > 0.1$). Therefore, only dietary treatment, as a main effect, is shown in this section.

Table 5. Total nitrogen (N) intake, ileal N and dry matter (DM) flows and true ileal N digestibility in pigs fed maize starch-based diets containing either wheat gluten, peas or rapeseed meal as the sole protein source (Experiment 1)

	Diet			RMSE[1]
	Wheat gluten	Peas	Rapeseed meal	
No. of observations	4	3	4	
Total N intake (g/d)	24.62	25.29	24.84	3.81
Ileal N flow (g/d)				
Total	2.19^{a}	4.26^{b}	7.63^{c}	0.76
Endogenous origin	1.81^{a}	3.10^{b}	2.67^{ab}	0.59
Feed origin	0.37^{a}	1.16^{a}	4.96^{b}	0.93
True ileal N digestibility (%)	98.6^{a}	95.6^{a}	80.1^{b}	2.15
DM flow (g/kg DMI)[2]	108.8^{a}	213.7^{b}	266.8^{c}	10.36
Ileal endogenous N (g/kg DMI)	2.10	3.66	3.05	0.90

[1] Root mean square of the error term; the degrees of freedom of the error term are 8 (11 observations).

[2] Dry matter intake.

$^{a, b, c}$ Means within a row lacking a common superscript letter differ at $P < 0.05$.

Apparent ileal digestibility

Digestibility values for DM and N differed ($P < 0.05$) among diets (Table 4). They were the highest for the WG diet, and the lowest for the RSM diet, with a difference of 16.6 percentage units for DM digestibility and of 24.2 percentage units for N digestibility.

True ileal nitrogen digestibility, and ileal flows of nitrogen and dry matter

The natural background ^{15}N-enrichments of N in the TCA-soluble blood plasma and the ingested feed were 0.3672 and 0.3670, respectively. The mean (± SE) estimated ^{15}N-enrichment excess for TCA-soluble blood plasma and ileal digesta N pools were 0.0227 (± 0.0022) and 0.0130 (± 0.0037), respectively.

The data on the mean endogenous flows (g/d and g/kg DMI), DM flow (g/kg DMI) and true ileal N digestibility are presented in Table 5. Pigs consumed daily about 25 g of N and there was no difference among treatments. Total N flow at the terminal ileum of pigs was lower for the WG diet than for the pea and RSM diets ($P < 0.05$). The total ileal N flows for pea and RSM diets were higher than for the WG diet by 2.1 and 5.4 units, respectively. The flow of ileal endogenous N (g/d) was the highest with the pea diet and the lowest with the WG diet ($P < 0.05$). It also tended to be higher than that measured with the RSM diet. The ileal flow of dietary N with the RSM diet was higher ($P < 0.05$) than those with either the WG or pea diets. Ileal endogenous N flow (g/kg DMI) in pigs did not differ and ranged from 2.10 to 3.66 and 3.05 g/kg DMI for the WG, pea and RSM diets, respectively. The true ileal digestibility of N for the WG and pea diets were higher ($P < 0.05$) than for the RSM diet by 18.1 and 15.5 percentage units, respectively. The DM flow with the WG, pea and RSM diets differed ($P \leq 0.05$) and increased from 109 with the WG diet to 214 and 267 g/kg DMI with the pea and RSM diets, respectively.

Table 6. Apparent faecal digestibilities of dry matter and nitrogen (N) in pigs fed maize starch-based diets, which supported different ileal endogenous N excretions and were balanced for apparent ileal digestible protein and amino acids (Experiment 2)

	Diet			RMSE[1]
	Wheat gluten	Peas	Rapeseed meal	
Apparent faecal digestibility (%)				
Dry matter	95.0^{a}	90.4^{b}	82.8^{c}	1.08
Nitrogen[2]	92.9^{a}	82.1^{b}	72.4^{c}	1.82

[1] Root mean square of the error term; the degrees of freedom of the error term are 27 (30 observations).

[2] Values corrected for the synthetic amino acids added into the diets, assuming ileal digestibility of synthetic amino acids of 100%.

[a, b, c] Means within a row lacking a common superscript letter differ at $P < 0.05$.

Apparent faecal digestibility

Apparent faecal digestibilities of DM and N differed ($P < 0.05$) among treatments (Table 6). They were the highest for the WG diet, intermediate for the pea diet, and the lowest for the RSM diet. The digestibility of DM and N with the RSM diet were 12.2 and 20.5 percentage units lower, respectively, than with the WG diet.

Table 7. Nitrogen partitioning in pigs fed maize starch-based diets, which supported different ileal endogenous N excretions and were balanced for apparent ileal digestible protein and amino acids (Experiment 2)

	Diet			RMSE[1]
	Wheat gluten	Peas	Rapeseed meal	
Mean body weight (kg)	23.28	22.96	22.82	1.45
N intake (g/d)	15.59^{a}	17.41^{b}	20.47^{c}	0.89
N faeces (g/d)	0.96^{a}	2.95^{b}	5.53^{c}	0.43
N absorbed[2] (g/d)	14.63	14.46	14.94	0.70
N ileal digestible[3]	14.25	14.01	13.80	0.68
N urine (g/d)	2.73^{a}	3.29^{b}	4.64^{c}	0.51
N fermented[4] (g/d)	0.20	0.26	1.04	
N urine corr.[5] (g/d)	2.53	3.03	3.60	
N retention (g/d)	11.90^{a}	11.17^{b}	10.30^{c}	0.58

[1] Root mean square of the error term; the degrees of freedom of the error term are 27 (30 observations).

[2] Calculated [N absorbed = N intake - N faeces].

[3] Estimated ileal digestible N available for N retention; calculated [N ileal digestible = N intake x Digestibility coefficient of N of the diet$_{\text{(Table 4)}}$ / 100].

[4] Estimated N excretion in urine related to the fermentation of undigested dietary protein in the large intestine; calculated from the difference of apparent faecal and ileal N digestibilities of the diets, which are presented in Tables 6 and 4, respectively. The values are corrected for the presence of synthetic amino acids in the diets (assumed 100% absorbable).

[5] Corrected N excretion in urine calculated from the difference between the total N excretion in urine and estimated N excretion related to the fermentation of undigested dietary protein in the large intestine (see [4]).

[a, b, c] Means in a row lacking a common superscript letter differ at $P < 0.05$.

Nitrogen balance

According to diet formulation, N intake was different ($P < 0.05$) in pigs fed the WG, pea and RSM diets (Table 7), with the lowest value for the WG diet and the highest for the RSM diet. The excretion of N in faeces increased ($P < 0.05$) from 0.96 g/d for the WG diet to 2.95 g/d for the pea diet and 5.53 g/d for the RSM diet. The calculated differences in absorbed N were very small and ranged from 14.63 to 14.46 and 14. 94 for the WG, pea and RSM diets, respectively. The excretion of N in urine of pigs fed the WG, pea or RSM diets increased ($P < 0.05$) from 2.73 to 3.29 and 4.64 g/d, respectively. The calculated amounts of urinary N resulting from a fermentation of undigested dietary protein in the large intestine increased from 0.20 g/d with the WG diet to 0.26 g/d with the pea diet and 1.04 g/d with the RSM diet. The differences in urinary N excretion corrected for the N fermented in the large intestine remained important. The N retention in pigs fed the corresponding diets decreased ($P < 0.05$) from 11.90 to 11.17 and 10.30 g/d, respectively.

The estimated ileal digestible amount of N did not differ ($P > 0.28$) among treatments [mean = 14.0 g/d (±0.12)]. The utilization of an estimated ileal digestible N for N retention differed ($P < 0.05$) among treatments (Figure 2). The highest estimated value was 83.5% for the WG diet compared to the pea diet (about 4 percentage units lower) and the RSM diet (8.5 percentage units lower).

Discussion

Ileal digestibilities and flows of dietary and endogenous nitrogen

The calculation of true ileal N digestibility of test feedstuffs was based on the ^{15}N-dilution technique. This was also used for measuring the flow of endogenous ileal N. Although validation of this technique is still the subject of a scientific discussion (De Lange et al., 1992; Moughan et al., 1992), it is considered a reasonable method for comparative evaluation of endogenous N flow at the terminal ileum of pigs fed protein containing diets (Huisman et al., 1992; De Lange et al., 1990; Schulze, 1994).

Apparent ileal digestibility of N in WG was lower by 3.6 percentage units than the Dutch tabular value (CVB, 1992), whereas the value for peas was 5.5 percentage units higher. The present value for peas is in agreement with the data reported by Huisman et al. (1992) and Le Guen (1993), who carried out their studies in similar conditions with pigs of comparable body weight, while the tabular value is based on a different number of both experiments and cultivars. The apparent N digestibility in RSM was similar to data reported for canola/rapeseed meal by De Lange et al. (1990) and Grala et al. (1994a), and differed from the tabular value (CVB, 1992), being about 3 percentage units lower.

The true and apparent digestibilities of N in WG differed by 7 percentage units. The true ileal digestibility of N in peas was almost as high as in WG, but the apparent ileal N digestibility was about 16 percentage units lower. The difference in apparent digestibilities resulted from the higher flow of endogenous N at the terminal ileum when feeding the pea-based diet. These results correspond to those of Huisman et al. (1992,

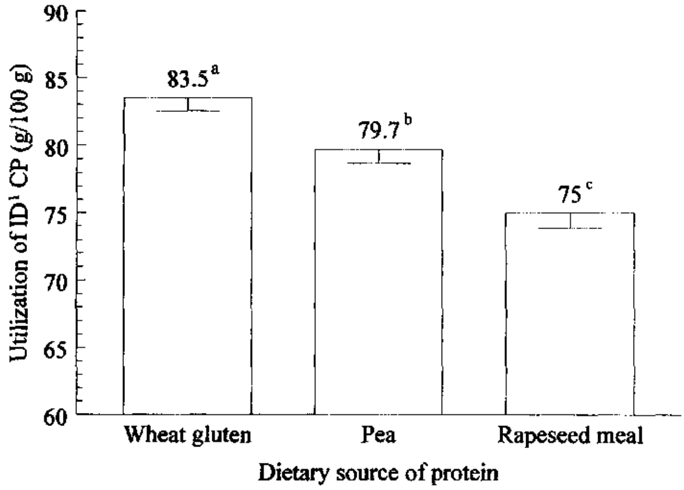

Figure 2. Utilization (%) of ileal digestible CP for N retention in pigs fed diets balanced for ileal digestible CP and amino acids, and containing wheat gluten, pea seeds or rapeseed meal with a low content of glucosinolates as protein source, which differ in excretions of ileal endogenous N (Experiment 1).

[1] Apparent ileal digestible.

[a, b, c] Means with different superscript letter differ at $P < 0.05$. Each value represents the mean of 10 pigs. The standard errors of the mean for diets with wheat gluten, peas, and rapeseed meal are 0.95, 1.01, and 1.14, respectively.

1993) and support their findings that a lower ileal apparent N digestibility of peas in pigs is mainly caused by a high flow of endogenous N at the terminal ileum.

Daily ileal flow of DM and endogenous N in pigs fed the pea diet were likely stimulated by the presence of antinutritional factors in peas, among which trypsin inhibitors are the most important (Leterme et al., 1990; Huisman and Le Guen, 1991; Huisman et al., 1992; Schulze, 1994). The increased ileal DM flow in pigs fed the pea diet can be associated with the presence of pea carbohydrates, notably the cell wall components. Leterme et al. (1994) found that pea inner fibre cause an increased output of the endogenous AA associated with a very high water holding capacity of these specific carbohydrates. As a result, the flow of ileal digesta can be increased (Jørgensen and Jensen, 1994). Bacterial N at the terminal ileum may partly be included in the endogenous N losses, and can be increased by the presence of pea carbohydrates (Le Guen, 1993). Schulze et al. (1994) calculated that even more than 50% of the total ileal N was of bacterial origin in pigs of similar BW fed semi-synthetic diets containing different levels of purified NDF. Since considerable quantities of ammonia and urea are secreted into the pig's small intestine, microbes can synthesize their own protein from these sources, in which ^{15}N is already incorporated (urea). As a result, the ^{15}N will contribute to the newly synthesized microbial protein and accounted as an essential part of endogenous N in ileal digesta (De Lange et al., 1992).

Both true and apparent N digestibilities of the RSM diet were relatively low at the terminal ileum. This was associated with a high flow of both endogenous N and undigested dietary N. These results agree with findings of de Lange et al. (1990) that considerable amounts of both feed and endogenous proteins are passing the distal ileum when pigs are fed rapeseed or canola meal.

The increased ileal flows of DM, undigested feed N, and endogenous N seem to be related to the high NDF content in rapeseed. These results agree with those of Schulze et al. (1995) who found that the level and source of NDF in the pig diet increase the ileal DM flow, as well as endogenous and undigested feed ileal N losses.

An important part of rapeseed fibre consists of lignin, pectin and hemicellulose. These components may interact with amino acids liberated during protein hydrolysis. In this way, some amino acids may escape absorption in the small intestine and pass into the large intestine (Bergner et al., 1981; Howard et al., 1986). A strong association of protein to the fibre fraction of rapeseed is likely to be the main reason for a negative effect on the true CP digestibility of rapeseed in rats (Bjergegaard et al., 1991). Thus, the increased flow of endogenous N at the terminal ileum of pigs could also be associated with the adsorptive properties of rapeseed fibre (Howard et al., 1986).

With RSM, there is a complex of factors that are related to the technological processes (e.g., temperature, time, humidity during toasting). The processing may negatively affect the true CP digestibility (studies with rats by Grala et al., 1994b), and the apparent ileal CP digestibility in pigs (Grala et al., 1994a). Furthermore, Batterham (1992) reported that with heat-treated feeds, due to advanced Maillard reactions, changes may occur to lysine and other amino acids. This may have only a small effect on the apparent digestibility of amino acids but may have a particular effect on their availability after absorption.

Nitrogen balance

The main objective of this study was to determine whether N retention is similar or not when pigs are fed diets that differ in endogenous protein losses, but are alike in the amounts of apparent ileal digestible protein and amino acids. It is generally accepted that only amino acids available and absorbed up to the end of the small intestine can be used for metabolism in the pig (e.g., N retention) (Żebrowska et al., 1978).

Results of Experiment 1 showed that low apparent ileal N digestibility of diets with peas or RSM was associated with a higher flow of ileal endogenous N compared to highly digestible WG. In Experiment 1, the diets were equal in CP content and balanced for ileal digestible amino acids. It was aimed to determine the apparent ileal digestibility of CP. In Experiment 2, the diets were balanced for both apparent ileal digestible CP and amino acids. The apparent ileal digestibility of protein in feedstuffs was assumed to be the same as in Experiment 1. If ileal digestibility of amino acids determines N balance, then a similar N retention should be expected in Experiment 2. Following this, it was hypothesized that eventual differences in N retention would be caused by a different urinary N excretion as induced by different flows of ileal endogenous N in pigs.

Indeed, although the diets provided similar amounts of the estimated apparent ileal digestible protein and amino acids, significant differences in N retention were found among treatments. The results showed that lower N retention with the pea and RSM diets than with the WG diet resulted from an increased excretion of urinary N. This also holds when the total urinary N was corrected for N that could appear in urine as a result of protein fermentation in the large intestine. Although the diets varied considerably in CP contents, the resulting different CP intakes and hindgut fermentation did not induce large differences in N retention. This was also shown by similar results on the utilization of ileal digestible N for N retention. So, these findings seem to support the hypothesis that an increased flow of ileal endogenous N may be associated with an increased excretion of N in urine and results in a lower N retention in the pig. The consequence, however, may be that the tabular values for the ileal amino acid digestibilities for most of essential amino acids could not indicate differences in their availability. Van Leeuwen et al. (1993) showed that there is a clear relationship between apparent ileal digestibility of CP and amino acids when the contents of CP and amino acids are analyzed in the feedstuff. Furthermore, the tabular DC values have been shown to be highly correlated with those calculated from the CP content in feedstuffs. Thus, the DC for lysine, methionine, cystine, threonine, and tryptophan of the feedstuffs could be obtained from tabular values with sufficient accuracy. If this holds for this study, a possible error in estimating the ileal digestible amino acid supply was relatively small and cannot explain the range of differences found in N balance among treatments. Nevertheless, it must be realized that the results of N balance are true when apparent ileal digestibilities of protein and amino acids were similar to those in Experiment 1, and the flow of ileal endogenous N was not affected by the way of diet formulation (e.g., variation in the CP contents of the diets in both experiments).

Generally, nitrogen excreted in ileal digesta originates from undigested feed protein and from undigested endogenous protein. Nitrogen in urine results from losses of N during metabolic processes, e.g., N losses occurring during protein turnover (Moughan, 1991, 1993) and from the absorbed end-products of fermentation in the intestinal tract.

The amount of endogenous N reabsorbed from the gut lumen was not determined in this study. Based on Souffrant (1991) and Krawielitzki et al. (1994), it was assumed that the reabsorption rate of secreted endogenous N was 75%. This means that the flow of endogenous N in the small intestine is four times higher than the flow at the terminal ileum. It seems unlikely that this ratio is constant for diets that, in practice, differ in chemical and physical characteristics. Therefore, this needs to be evaluated in further studies. As discussed earlier, the results strongly suggest that N retention may be decreased when the flow (loss) of ileal endogenous N is increased. Nevertheless, the cause of the extra urinary N loss, which produces the factual differences in N retention, cannot be established without data on reabsorption rate of endogenous N. Losses of endogenous gut protein *per se* do not fully explain why the post-absorptive (in)efficiency of protein utilization differ among diets. Since the utilization of ileal digestible N was decreased with an increased loss of ileal endogenous N, it might be that, due to this loss, the amino acid requirement for maintenance was increased. In such a case, a preferential choice of the pig was to supply amino acids for endogenous protein (re)synthesis at the expense of protein synthesis for body gain. Moughan (1991) indicated that maintenance processes have a priority for nutrients over growth processes.

Furthermore, since dietary amino acids were balanced to supply ideal protein for growth, but were partly used for endogenous purposes, it might be considered that this will change the balance of the total amino acid pool and result in increased amino acid oxidation and urea synthesis. As a result, urinary N excretion will be increased. Especially, this is of importance when extra endogenous protein losses caused by dietary factors, e.g., protease inhibitors and(or) fibre, become critical for amino acid balance.

Considering limited data from the N balance study and its limitations, it may be supposed, however, that the higher amount of recycled endogenous amino acids the more altered will be the free amino acid pattern in the precursor pools for body protein synthesis and the less efficient these pools may become for protein deposition. This will result in increased oxidation and induce inefficiency in N utilization. More specific studies are needed for that, since the present studies could provide only relative data about this matter.

Conclusions

This study confirmed that urine is an important route of N loss and it may negatively affect N balance in the pig. This N loss can be dependent on the characteristics and nature of dietary protein sources, e.g., their influence on ileal endogenous protein losses. However, the present study, due to its limitations, could not give an answer on the character, origin and site(s) of urinary N loss as dependent on ileal endogenous N losses. Further studies on this topic are scheduled. The results imply that for validating the nutritional value of feedstuffs for pigs not only apparent digestibility of amino acids is of importance but also losses of endogenous protein caused by the feedstuffs.

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CHAPTER 2

ILEAL APPARENT PROTEIN AND AMINO ACID DIGESTIBILITIES AND ENDOGENOUS NITROGEN LOSSES IN PIGS FED SOYBEAN AND RAPESEED PRODUCTS

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Ileal Apparent Protein and Amino Acid Digestibilities and Endogenous Nitrogen Losses in Pigs Fed Soybean and Rapeseed Products

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ABSTRACT

We investigated the effects of various protein sources on the apparent ileal digestibilities (AID) of CP and amino acids (AA) and on the recoveries of ileal endogenous nitrogen (N) in pigs. Ileal endogenous N losses (ENL) were measured using the ^{15}N-isotope dilution method. Thirteen pigs (BW of 13 to 20 kg) were fitted with a post-valve T-cecal cannula and two indwelling blood catheters. They were fed twice daily at a level of 2.6 times ME for maintenance. Cornstarch-based diets contained a soy concentrate (SC; 180.5 g/kg), soybean meal (SBM; 295 g/kg), or a mixture of toasted and untoasted soybean meal (mSBM; 330.4 g/kg) in Trial I, or three rapeseed cakes, dehulled-toasted (RC1; 395 g/kg), non-dehulled-toasted (RC2; 458 g/kg) and dehulled-untoasted (RC3; 390 g/kg) in Trial II. The protein sources provided diets with similar levels of apparent ileal digestible CP (108 g/kg as-fed diet) and Lys, Met+Cys, Thr, and Trp. The AID of CP was greater ($P < .05$) for the SC (86.8%) and SBM (82.8%) than for the mSBM (68.1%) diet. In Trial II, the AID of CP was greater ($P < .05$) for RC! (76.2%) and RC3 (75.8%) than for the RC2 (69.5%) diet. For all diets, the differences in the AID for most of the AA corresponded with the differences in the AID of CP. The ENL (g/kg DMI) were greater ($P < .05$) for the mSBM diet (3.75) than for the SC (2.15) and SBM (2.53) diets in Trial I but were similar ($P > .05$; 2.24, 3.03, 2.89 for RC1, RC2, and RC3, respectively) among diets in Trial II. We concluded that AID of CP of the soybean diets were associated with endogenous and dietary N losses. For these diets, increased ENL and dietary N losses were associated with a higher dietary trypsin inhibitor activity. For the rapeseed diets, dehulling increased AID of CP and AA, due to reduced ENL ($P = .08$) and dietary N losses ($P < .05$). Toasting of dehulled rapeseed cake did not affect the AID of CP and AA ($P > .05$) while reducing the true ileal CP digestibility ($P < .05$).

Key Words: Pigs; Nitrogen; Digestibility; Soybeans; Rapeseed; Feed Processing

Introduction

Determinations of apparent ileal digestibilities in feedstuffs provide better estimates for amino acids availabilities in pigs than do fecal measurements (reviews by Sauer and Ozimek, 1986, and Tanksley and Knabe, 1993). Studies of de Lange et al. (1990), Huisman et al. (1992), and Schulze (1994) showed that differences in apparent ileal digestibility of protein among feedstuffs are mostly related to variations in endogenous protein recovered in ileal digesta. The ileal digesta of pigs contain undigested feed and endogenous proteins. Important sources of endogenous proteins are saliva, gastric juice, pancreatic juice, bile, intestinal juices, mucosa, and sloughed mucosal cells (Souffrant, 1991). The level of endogenous nitrogen (N) in ileal digesta of pigs is influenced by animal factors such as age and body weight (Mariscal-Landin et al., 1995) and by dietary factors. The latter are DMI (Butts et al., 1993), level and

Table 1. Chemical characteristics of tests products (g/kg DM)[a]

	SC	SBM	uSBM	RC1	RC2	RC3
DM	953.0	872.0	910.0	959.0	947.0	937.0
CP (N × 6.25)	709.3	529.8	578.0	385.8	356.9	386.3
Ether extract	8.4	21.7	27.5	205.4	122.5	202.8
Ash	72.4	71.1	68.1	71.9	71.8	71.5
Crude fiber	29.4	51.6	27.5	119.9	153.1	112.1
NDF	ND	87.2	62.6	149.1	241.8	156.9
TIA, mg/g[b]	2.5	5.5	35.1	ND	ND	ND
GLS, µmol/g of fDM[c]	ND	ND	ND	17.8	12.6	25.3
Amino acids						
Arg	47.6	38.6	41.6	24.3	21.1	24.8
His	19.8	15.5	14.5	10.8	9.2	11.1
Ile	33.2	24.5	28.4	16.2	14.9	17.2
Leu	57.9	42.2	44.0	28.2	25.8	29.4
Lys	46.5	36.2	37.0	19.8	18.6	21.0
Met	10.3	7.6	8.7	7.4	6.4	8.8
Cys	11.1	9.1	9.2	8.9	8.1	8.8
Phe	37.1	28.9	28.4	16.2	12.1	16.8
Thr	27.3	22.6	24.3	17.5	17.3	18.1
Trp	9.2	6.9	7.5	5.2	4.6	5.3
Val	35.1	27.3	28.9	21.2	21.1	23.3
Ala	31.2	24.7	24.8	18.6	16.8	18.7
Asp	84.8	66.4	69.3	30.1	27.6	30.6
Glu	127.4	99.4	108.7	65.6	58.1	66.5
Gly	31.5	23.6	24.3	20.9	19.1	21.4
Pro	45.3	35.6	31.8	22.0	21.8	21.4
Ser	35.7	30.0	32.4	18.9	18.0	19.1
Tyr	25.7	18.6	20.2	12.7	11.8	13.4

[a] SC = soybean concentrate; SBM = toasted soybean meal; uSBM = untoasted soybean meal; RC1 = dehulled-toasted rapeseed cake; RC2 = non-dehulled-toasted rapeseed cake; RC3 = dehulled-untoasted rapeseed cake; ND = not determined.

[b] Trypsin inhibitor activity (TIA) expressed as milligrams of trypsin inhibited per gram of dry matter.

[c] Glucosinolates (GLS) expressed as micromoles per gram fat-free dry matter.

quality of dietary protein (de Lange et al., 1990; Butts et al., 1993), and presence of antinutritional factors, such as trypsin inhibitors, lectins (Schulze, 1994), and tannins (Jansman et al., 1995). Also, dietary fibers are associated with ileal endogenous N losses (**ENL**; Leterme et al., 1994; Schulze et al., 1995).

The present investigation was undertaken to study the effects of differently processed soybean and rapeseed products, as the only protein sources in semisynthetic diets, on the apparent digestibilities of protein and amino acids and on the recovery of endogenous N at the terminal ileum of pigs.

Materials and Methods

Animals and Housing. The experiment was carried out using 13 (Dutch Landrace × Yorkshire) × Finnish Landrace barrows. Pigs were about 6 wk old and had a BW (± SE) of 9.2 kg (± .28 kg) upon arrival. They were individually housed in metabolism cages (80 × 180 cm) at an ambient temperature of 22 to 24°C and a relative air humidity of approximately 55%. The animals could move freely in the cages. Before the first experimental period, pigs were randomly allocated to six dietary treatments. The investigation was reviewed and approved by the Netherlands Organization for Applied Scientific Research (TNO) Committee for Animal Welfare.

Feedstuffs. Three soybean and three rapeseed products were used in two trials of the same investigation (Table 1). In Trial I, the soybean products were a commercial soybean concentrate (**SC**), which was used as a reference protein source within Trial I, dehulled-toasted soybean meal (**SBM**), and dehulled-untoasted soybean meal (**uSBM**).

For preparation of SBM and uSBM, soybeans were conditioned at a temperature of 65°C for 30 min, and then for 15 min at 75°C. Then, the beans were crushed, dehulled, flaked, and extracted with hexane at 50 to 55°C. The uSBM was prepared in a vacuum desolventizer-deodorizer; flakes were subsequently treated for 2 to 3 min at 50 to 55°C and a pressure of 80 to 90 kPa, then for 4 to 5 min at 70 to 75°C and a pressure of 95 kPa. Moisture was regulated by live steam injection (12 $L{\cdot}t^{-1}{\cdot}h^{-1}$). The SBM was prepared in a desolventizer-toaster, where the flakes stayed for 6 to 7 min at 75°C. Then flakes were toasted at 105°C for 25 min. Moisture during toasting was regulated by steam injection (50 to 60 $L{\cdot}t^{-1}{\cdot}h^{-1}$).The SC was prepared from untoasted, defatted flakes, which were extracted with acetone/water (70/30, vol/vol) at 70 to 72°C for 150 to 180 min. Then the product was toasted for 25 min at 105°C.

In Trial II, the rapeseed products (defatted cakes) were made from dehulled-toasted (**RC1**), non-dehulled-toasted (**RC2**), and dehulled-untoasted (**RC3**). The defatted cakes were made from one batch of rape seed. They were prepared by CETIOM-GERDOC (Pessac, France) from rape oilseeds (*Brassica napus* L.) of the low glucosinolate French variety Marmandes (canola type).

Table 2. Ingredient composition of the experimental diets (%)

	Trial I			Trial II		
Item	SC	SBM	mSBM	RC1	RC2	RC3
Soybean concentrate	18.05	-	-	-	-	-
Toasted soybean meal	-	29.50	24.78	-	-	-
Untoasted soybean meal	-	-	8.26	-	-	-
Dehulled-toasted rapeseed	-	-	-	39.50	-	-
Non-dehulled-toasted	-	-	-	-	45.80	-
Dehulled-untoasted	-	-	-	-	-	39.00
Cornstarch	52.10	45.01	43.11	40.59	32.35	41.12
Dextrose	15.00	15.00	15.00	15.00	15.00	15.00
Soybean oil	1.30	1.30	1.30	-	2.50	-
Cellulose	8.00	4.50	3.00	-	-	-
Chalk	1.16	1.18	1.20	.88	.77	.90
Monocalcium phosphate	1.80	1.70	1.65	1.50	1.35	1.50
NaCl	.30	.30	.30	.30	.30	.30
$KHCO_3$	.75	.10	-	.75	.45	.75
$NaHCO_3$	.25	.25	.25	.25	.25	.25
MgO	.10	-	-	-	-	-
Mineral-vitamin mixture[a]	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine	.02	.03	.02	.22	.22	.17
L-Threonine	.01	.01	.01	-	-	
DL-Methionine	.15	.11	.11	-	-	-
L-Tryptophan	.01	.01	.01	.01	.01	.01

[a] The premix supplied per kilogram of feed: 9,000 IU of vitamin A; 1,800 IU of vitamin D_3; 40 mg of vitamin E; 5 mg of riboflavin; 30 mg niacin; 12 mg of d-pantothenic acid; 1,000 mg of choline, 40 µg of vitamin B_{12}; 2 mg of thiamine; 3 mg of pyridoxin; .1 mg of biotin; 1 mg of folic acid; 3 mg of vitamin K; 50 mg of ascorbic acid; 72.8 mg of Zn ($ZnSO_4 \cdot H_2O$); 44 mg of Mn (MnO_2); 80 mg of Fe ($FeSO_4 \cdot 7H_2O$); .525 mg of Co ($CoSO_4 \cdot 5H_2O$); .38 mg of K (KI); .254 mg of Cu ($CuSO_4 \cdot 5H_2O$); .06 mg of Se ($Na_2SeO_3 \cdot 5H_2O$); 40 mg tylosin.

For manufacturing of RC2, seeds were flaked, cooked at 80 to 85°C for 45 min, and the oil was removed by continuous pressing with a screw (MBU) press. In a desolventizer-toaster, the cake was heated until 100°C, toasted at this temperature for 10 min, then cooled for 20 min to 180°C (live steam injection at 50 kg/h until the beginning of cooling [25 min]).

For processing RC1 and RC3, part of the same batch of seeds used for preparation of RC2 was dehulled in a dehuller-sorter. The hulls were separated from kernels on a fluidized bed sorter. The kernels coming out from the sorter were purified on a cleaner with a 1-mm screen. The oil separation from the kernels was processed by double pressing. First pressing of whole kernels was in a continuous type screw press (S10) after cooking for 30 min at approximately 60°C. The flaked kernels coming from the S10 press were pressed in the MBU press. One part of this cake was used as RC3 (dehulled-untoasted), and the second part was toasted as described earlier, yielding the dehulled-toasted cake (RC1).

Diets and Feeding. The soybean and rapeseed products were the only protein sources in diets (Table 2). The SBM and uSBM were mixed in a ratio of 75:25, and the diet was coded as **mSBM diet**. The other two soybean diets and three rapeseed diets were coded as **SC diet** and **SBM diet**, **RC1 diet**, **RC2 diet**, and **RC3 diet**. These names refer to the codes of the products. In both trials, the diets contained 108 g/kg diet of calculated apparent ileally digestible (**ID**) CP (N × 6.25). Apparent ileal digestibility (**AID**) values for CP of the test products were estimated in a previous trial (our unpublished observations). The AID coefficients for CP in SC, SBM, mSBM, RC1, RC2, and RC3 were 87, 78, 68, 72, 68, and 75%, respectively. The content of apparent ID lysine, threonine, methionine, tryptophan, and the sum of methionine and cystine were at least 75% of the requirement values for pigs of 10 to 25 kg BW (CVB, 1994) and are comparable within Trials I and II. The diets were supplemented with crystalline amino acids (**AA**) to accomplish an optimum ratio of first-limiting AA to lysine (CVB, 1994) and to ensure appropriate growth of pigs. Chromic oxide (Cr_2O_3) was added to the diets as an indigestible marker (2.5 g/kg as-fed diet). Analyzed and calculated contents of nutrients in the diets are given in Table 3.

During the experimental period, diets were fed twice a day in equal meals at 0800 and 2000. Pigs were individually fed at a level of 2.6 times the maintenance requirement for ME (2.6 x 420 kJ ME/kg$^{.75}$ BW; ARC, 1981). The BW of pigs were determined and feeding levels adjusted at d 1, 10, and 19 of the experimental period (Figure 1). Feed was mixed with water (1:2, wt/vol) just before feeding. No additional water was supplied.

Table 3. Chemical composition (g/kg as-fed) of the experimental diets[a]

Item	Trial I			Trial II		
	SC	SBM	mSBM	RC1	RC2	RC3
Chemical composition (analyzed)						
DM	902.2	903.8	904.7	913.9	915.6	911.3
CP (N × 6.25)	121.9	141.4	163.4	144.8	154.1	138.7
Ether extract	14.7	18.9	20.1	78.8	78.8	74.5
Crude fiber	88.6	62.6	47.5	46.9	67.6	46.3
Ash	50.7	52.4	53.9	58.9	58.9	57.9
NDF	ND	ND	ND	84.1	116.4	79.4
TIA[b]	.41	1.47	3.53	ND	ND	ND
Essential amino acids as estimated[c]						
Lys	8.1	9.0	10.4	9.3	9.8	9.1
Met	3.1	3.2	3.5	2.8	2.8	3.3
Met+Cys	4.9	5.4	6.1	6.2	6.4	6.5
Thr	5.3	5.8	6.8	6.8	7.6	6.7
Trp	1.7	1.9	2.2	2.1	2.1	2.0
Apparent ileal digestible (ID) CP and amino acids as estimated[d]						
ID CP (N × 6.25)	108.0	108.0	108.0	108.0	108.0	108.0
ID Lys	7.5	7.5	7.5	7.5	7.5	7.5
ID Met	3.0	2.8	2.8	2.4	2.3	2.8
ID Met+Cys	4.4	4.4	4.4	4.9	4.8	5.4
ID Thr	4.4	4.4	4.4	4.5	4.8	4.7
ID Trp	1.5	1.5	1.5	1.5	1.5	1.5
ME, MJ/kg[e]	14.4	14.4	14.4	14.4	14.4	14.4

[a] SC = soybean concentrate; SBM = toasted soybean meal; mSBM = mixture of toasted and untoasted soybean meal; RC1 = dehulled-toasted rapeseed cake; RC2 = non-dehulled-toasted rapeseed cake; RC3 = dehulled-untoasted rapeseed cake.

[b] Trypsin inhibitor activity (TIA) expressed as milligrams of trypsin inhibited per gram of air-dry matter.

[c] Amino acid contents in the diets were calculated from the analyzed contents in the protein sources.

[d] ID CP and ID AA were estimated based on the calculated levels of CP and AA that supplied the diets and then corrected for the apparent ileal digestibility values.

[e] ME was calculated from its content in the ingredients (CVB, 1994).

Preliminary period				Experimental period						
1-12	13-14	15-20	21-22	1-6	7-9	10-15	16-18	19-24	25-27	28
Adaptation	PVTC-cannulation	Recovery	Catheters	Pre-period 1	Digesta collection 1	Pre-period 2	Digesta collection 2	Pre-period 3	Digesta collection 3	The end
				[^{15}N]leucine infusion						

Figure 1. Scheme of the experimental procedure. PVTC = ileal cannulation using post-valve-T-cecum cannula (van Leeuwen et al., 1991). Catheters = insertion of two blood catheters into blood vessels, the firs one placed into the external jugular vein for blood sampling and the second one into the carotid artery for the infusion of [^{15}N]leucine.

Experimental Procedure. The experimental scheme is presented in Figure 1. After an adaptation period on a commercial diet (12 d), 12 pigs were surgically fitted with a post-valve-T-cecum cannula (van Leeuwen et al. 1991). The recovery period (7 to 8 d) was followed by the insertion of two catheters into blood vessels (van Leeuwen et al., 1995). The first catheter was placed into the external jugular vein for blood sampling, and the second into the carotid artery for the infusion of [^{15}N]leucine.

Pigs were used in a changeover arrangement of treatments with three periods of 9 d each. In each period, there were 6 d for adaptation to the dietary treatments and three successive days of digesta collections from 0800 to 2000. Ileal digesta were collected as described by Schulze et al. (1995). The first period started with the simultaneous introduction of experimental diets and with the [^{15}N]leucine infusion. A continuous 27-d infusion of [^{15}N]leucine started 1-d after catheterization. The infusion rate was 4.2 mg of [^{15}N]leucine (95% ^{15}N enrichment)$\cdot$kg^{-1} of BW$\cdot$d^{-1}. The [^{15}N]leucine (Euro-Top, Group CEA, Saint-Aubin Cedex, Belgium) was dissolved in a sterile nonpyrogenic, physiological saline solution (NaCl, 9 g/L) and infused with perfusion pumps (F. Braun Melsungen AG, Germany). The "swivel technique" was used to infuse the freely moving pigs continuously (van Kleef, 1996). Blood samples of approximately 8 mL were taken during the digesta collections at 1400 each day. After sampling, blood samples were treated as described by Schulze et al. (1995).

Chemical Analyses. Samples of ileal digesta were pooled per pig within each experimental period. Nitrogen, DM, and ether extract (**EE**) in the diets and in freeze-dried ileal digesta were analyzed according to standard methods (AOAC, 1984). Amino acids were analyzed following the method of Andrews and Baldar (1985). Neutral detergent fiber was determined as described by Englyst and Cummings (1988). Chromium in the diets and ileal digesta was determined according to Bosch et al. (1988). The soy products and diets were analyzed for trypsin inhibitor activity (**TIA**) according to van Oort et al. (1989). Glucosinolates were determined according to the method 1864/90 of EEC (1990). The ^{15}N-enrichment of ileal digesta, feed, and of the trichloroacetic acid (**TCA**)-soluble fraction of blood plasma was determined according to the procedure described by Schulze et al. (1995).

Data Analyses. The AID for DM, CP and AA were determined using the chromic oxide ratio method (Marty et al., 1994). For the AID calculations of CP and AA, corrections have been made for N added to the diets in the form of crystalline AA. These AA were assumed to be 100% absorbed before the end of the ileum. The ratio of ^{15}N-enrichment excess in ileal digesta to that in the TCA-soluble blood plasma was determined. From that ratio the proportion of endogenous N in ileal digesta was estimated using the following formula (de Lange et al., 1990):

$$N_e = N_d \times [(^{15}N_d - {}^{15}N_{diet(0)}) / (^{15}N_{pl} - {}^{15}N_{pl(0)})]$$

where N_e is endogenous N in ileal digesta, N_d is total N in ileal digesta, $^{15}N_d$ is ^{15}N-enrichment of ileal digesta (%), $^{15}N_{diet(0)}$ is natural ^{15}N-enrichment of the diet (%), $^{15}N_{pl}$ is ^{15}N-enrichment in the TCA-soluble blood plasma (%), and $^{15}N_{pl(0)}$ is natural ^{15}N-enrichment in the TCA-soluble blood plasma (%). The true ileal CP digestibilities of the diets were calculated by correcting the AID coefficients for the ileal endogenous CP (N x 6.25). At the beginning of the experimental period (start of the ^{15}N infusion), the average BW of pigs was 13.0 kg (± .21 kg). By the end of the experiment, the average BW was 20.1 kg (± .31 kg).

Originally, the arrangements of the experiment ensured six replications of each treatment within both Trial I and Trial II. In Trial II, because of dysfunction of the jugular vein catheter, one animal (RC3 diet in period 1) was replaced by another one of similar genetic background and BW (this observation was treated as a missing value). In period 1, two pigs fed the mSBM diet and one pig fed the SBM diet, and in periods 2 and 3 one pig fed the RC3 diet did not maintain the required feeding level throughout the experimental periods. Data for these pigs were also treated as missing values. The total numbers of observations were six for the SC, RC1, and RC2 diets, five for the SBM diet, four the mSBM diet, and three for the RC3 diet. Consequently, the design of the trials was not balanced as originally scheduled. As a result, the period effect was not included in the statistical model. It was assumed that, similarly to the previous study (Grala et al., 1997), the period effect would be absent. The animal effect was not significant (P > .10); thus the results were subjected to the GLM procedure (SAS, 1990) with treatment (experimental diet) as a main factor and BW of the pig as a covariable for the treatment according to the following model for each trial:

$$Y_{ij} = \mu + T_i + \beta \times BW_{ij} + e_{ij}$$

where Y_{ij} is dependent variable, μ is overall mean, T_i is treatment (Trial I: i = 1, 2, 3 or Trial II: i = 1, 2, 3), BW_{ij} is average BW of individual pigs (j = 1, 2 ... 13) during each digesta collection, and e_{ij} is residual error. The differences among treatments were determined for the leastsquares means (LSM) using multiple comparisons of difference (PDIFF) with adjustment for the Tukey-Kramer test to detect the significance at $P < .05$ (SAS, 1990). The correlation coefficients between the TIA level in the diets, or the NDF content in the diets and the experimental variables were evaluated using the CORR procedure of SAS (1990).

Results

Although pigs remained healthy, some feed refusals were recorded during the experiment. The feed refusals were most likely related to poor palatability of the mSBM and RC3 diets. The CP content in the diets ranged from 121.9 to 163.4 g/kg (as-fed basis; Table 3). The inclusion of soybean products differing in TIA levels resulted in .41, 1.47, and 3.90 mg of trypsin inhibited per gram of the SC, SBM, and mSBM diets, respectively. In RC1 and RC3, dehulling decreased the average NDF content by 37% and increased the CP content by 8% as compared with the non-dehulled RC2 (Table 3). The inclusion of dehulled rapeseed products in the diets resulted in lower NDF contents compared with the RC2 diet. The average AA content and concentration of glucosinolates in RC1 after dehulling increased by 10 and 41%, respectively, compared with RC2 (Table 1). Toasting did not affect the nutrient contents (RC3 vs RC1) but decreased the glucosinolate content by 30% in toasted RC1 compared with untoasted RC3. The content of EE ranged from 122.5 (RC2) to 205.5 g/kg DM (RC1).

Apparent Ileal Digestibility of Dry Matter, Crude Protein and Amino Acids

Trial I. Apparent ileal digestibilities (AID) of DM and CP for the SBM or mSBM diets were lower than for the SC diet ($P < .05$) (Table 4). The mSBM diet had almost 19 percentage units lower AID of CP than the SC diet. The mSBM diet had

Table 4. Apparent ileal digestibility (%) of DM, CP, and amino acids (AA) of the cornstarch-based diets containing either soybean or rapeseed products[a] as the sole protein source[b]

	Trial I				Trial II			
Item	SC	SBM	mSBM	RMSE[c]	RC1	RC2	RC3	RMSE
DM	82.9[d]	81.2[e]	79.9[f]	.7	80.6[d]	74.6[e]	80.5[d]	.8
CP (N × 6.25)	86.8[d]	82.8[d]	68.1[e]	2.6	76.2[d]	69.5[e]	75.8[d]	2.2
Arg	95.3[d]	92.5[d]	79.7[e]	2.0	87.1[d]	83.8[e]	85.9[de]	1.8
His	86.7[d]	82.7[e]	69.5[f]	2.6	78.9[d]	73.3[e]	80.0[d]	1.4
Ile	91.2[d]	87.0[e]	68.6[f]	2.1	80.5[d]	74.9[e]	79.0[d]	1.9
Leu	90.7[d]	86.6[e]	68.5[f]	2.3	82.2[d]	79.3[d]	80.2[d]	2.0
Lys	92.9[d]	90.6[d]	80.3[e]	1.5	80.1[d]	72.6[e]	82.1[d]	2.2
Met	91.4[d]	88.7[d]	77.3[e]	2.6	86.4[d]	85.4[d]	86.6[d]	1.4
Cys	81.7[d]	77.8[d]	64.9[e]	2.8	79.6[d]	70.8[e]	79.6[d]	2.3
Phe	91.5[d]	88.0[d]	70.0[f]	2.3	82.9[d]	79.2[e]	80.8[de]	1.8
Thr	80.9[d]	79.3[d]	66.4[e]	2.2	76.6[d]	67.3[e]	74.8[d]	2.4
Trp	87.6[d]	83.0[e]	68.6[f]	1.5	81.6[d]	74.8[e]	79.9[d]	2.3
Val	88.2[d]	84.2[d]	67.6[e]	2.4	80.7[d]	75.4[e]	79.1[d]	1.8
Ala	86.2[d]	82.3[d]	68.1[e]	2.4	79.7[d]	75.1[e]	78.0[de]	2.3
Asp	86.5[d]	86.7[d]	71.1[e]	2.2	78.8[d]	72.1[e]	77.7[d]	2.0
Glu	92.7[d]	90.1[d]	76.1[e]	2.5	84.9[d]	81.8[e]	85.1[d]	1.8
Gly	80.9[d]	78.0[e]	65.0[f]	1.6	78.5[d]	70.1[e]	75.2[d]	3.4
Pro	87.4[d]	85.4[d]	70.0[e]	2.0	77.3[d]	67.9[e]	78.8[d]	3.8
Ser	88.1[d]	84.5[e]	69.6[f]	2.1	78.5[d]	72.7[e]	77.3[d]	1.9
Tyr	91.2[d]	87.6[d]	70.9[e]	2.2	82.5[d]	76.0[e]	80.2[d]	2.0
Sum AA	89.4[d]	86.7[d]	71.8[e]	2.0	81.1[d]	75.8[e]	80.5[d]	2.0

[a] SC = soybean concentrate; SBM = toasted soybean meal; mSBM = mixture of toasted and untoasted soybean meal; RC1 = dehulled-toasted rapeseed cake; RC2 = non-dehulled-toasted rapeseed cake; RC3 = dehulled-untoasted rapeseed cake.

[b] Digestibility coefficients of CP and AA were corrected for N added as crystalline AA, assuming their absorption of 100% before the end of the ileum.

[c] Root of mean square error (df = 14 within Trials I and II).

[d,e,f] Means for each trial within a row lacking a common superscript letter differ ($P < .05$).

a lower AID for total AA compared with the SC or SBM diets ($P < .05$). Differences in the digestibility values between the SC diet and the mSBM diet ranged from 13 percentage units (lysine) to 23 percentage units (isoleucine). The SBM diet had intermediate digestibility values. The TIA levels in the diets were negatively correlated with the AID of DM, CP, and the sum of AA ($r = -.86$, $P < .01$; $r = -.95$, $P < .01$; $r = -.95$, $P < .01$, respectively).

Trial II. The diets with toasted (RC1) and untoasted (RC3) dehulled rapeseed cakes had higher ($P < .05$) AID for DM and CP than the diet with non-dehulled-toasted rapeseed cakes (RC2; Table 4). For the RC1 or RC3 diets, digestibility values for DM were six percentage units higher than for the RC2 diet. Also for the RC1 and RC3 diets, digestibility values of CP were about seven percentage units higher than for the RC2 diet. Apparent ileal digestibilities for all AA were similar for the RC1 and the RC3 diets ($P > .05$). Both diets had higher AID for all AA than the RC2 diet ($P < .05$), except leucine and methionine for the RC1 vs RC2 diets, and arginine, phenylalanine, alanine, and glycine for the RC3 vs RC2 diets. The contents of NDF in the diets were negatively correlated with the AID of DM, CP, and the sum AA ($r = -.97$, $P < .01$; $r = -.85$, $P < .01$; $r = -.81$, $P < .01$, respectively).

Table 5. ^{15}N-enrichment excess[a] in the TCA-soluble blood plasma and ileal digesta of pigs fed cornstarch-based diets containing either soybean or rapeseed products[b] as a sole protein source

	Trial I				Trial II			
Item	SC	SBM	mSBM	RMSE[c]	RC1	RC2	RC3	RMSE
	^{15}N-enrichment excess							
Blood plasma[d]	.0321	.0287	.0290	.0035	.0289	.0292	.0262	.0023
Ileal digesta	.0231[e]	.0170[f]	.0120[g]	.0014	.0108[e]	.0107[e]	.0127[e]	.0019

[a] The background ^{15}N-enrichment of the N in TCA-soluble blood plasma and feed were .3663 and .3665, respectively.

[b] SC = soybean concentrate; SBM = toasted soybean meal; mSBM = mixture of toasted and untoasted soybean meal; RC1 = dehulled-toasted rapeseed cake; RC2 = non-dehulled-toasted rapeseed cake; RC3 = dehulled-untoasted rapeseed cake.

[c] Root of Mean Square Error (df = 14 both within Trial I and II).

[d] TCA-soluble blood plasma.

[e,f,g] Means for each trial within a row lacking a common superscript letter differ ($P < .05$).

^{15}N enrichment of Blood Plasma and Ileal Digesta

The background ^{15}N-enrichment in total N in TCA-soluble blood plasma and feed were .3663 and .3665%, respectively. The ^{15}N-enrichment excess for the TCA-soluble blood plasma, corrected for the BW effect ($P < .05$), did not differ ($P > .05$) among the soybean and rapeseed treatments (Table 5). The ^{15}N-enrichment excess in the ileal digesta, corrected for the BW effect ($P < .01$), was different for the SC, SBM and mSBM diets ($P < .01$). For the soybean diets, the TIA levels were negatively correlated with the ^{15}N-enrichment excess of the ileal digesta ($r = -.86$, $P < .01$). For the rapeseed diets, the ^{15}N-enrichment excess in the ileal digesta, corrected for the BW effect ($P < .05$), did not differ among rapeseed diets ($P > .05$).

Table 6. Flow of DM and recoveries of total nitrogen (N), endogenous N, and dietary N in addition to the true digestibility of CP at the terminal ileum of pigs as affected by various soybean products[a] as a sole protein source in the cornstarch-based diets (Trial I)

	Diet(g TIA[b]/kg DM of diet)			
Item	SC (.45)	SBM (1.62)	mSBM (3.90)	RMSE[c]
No. of observations	6	5	4	-
Ileal DM flow, g/kg of DMI	171.1^{f}	187.7^{e}	201.2^{d}	7.22
Nitrogen in ileal digesta				
Total, g/kg of DMI	2.81^{f}	4.27^{e}	9.19^{d}	.71
Endogenous, g/kg of DMI	2.15^{e}	2.53^{e}	3.75^{d}	.46
Dietary, g/kg of DMI	.66^{e}	1.74^{e}	5.44^{d}	.80
Endogenous N, g/g of total N	.76^{d}	.59^{e}	.42^{f}	.09
True ileal CP digestibility, %	96.9^{d}	93.0^{d}	81.1^{e}	2.89

[a] SC = soybean concentrate; SBM = toasted soybean meal; mSBM = mixture of toasted and untoasted soybean meal; RC1 = dehulled-toasted rapeseed cake; RC2 = non-dehulled-toasted rapeseed cake; RC3 = dehulled-untoasted rapeseed cake.

[b] Trypsin inhibitor activity.

[c] Root of mean square error (df = 14).

[d,e,f] Means within a row lacking a common superscript letter differ ($P < .05$).

Dry Matter Flow and Recoveries of Total, Endogenous, and Dietary Nitrogen at the Terminal Ileum

Trial I. The use of the mSBM diet (high TIA) increased the flow of ileal DM compared with the SBM and SC diets ($P < .05$; Table 6). The flow of ileal DM for the SBM diet was higher than for the SC diet ($P < .05$). The recovery of total ileal N was different for the SC, SBM and mSBM diets ($P < .05$). For the SBM and mSBM diets, the recoveries of total ileal N increased by 50 and 220%, respectively, compared with the SC diet. These differences resulted from higher losses of endogenous N and dietary N ($P < .05$). Consequently, the ratio of endogenous N to total N in ileal digesta was markedly higher ($P < .05$) for the SC diet (.76 g/g) than for the SBM diet (.59 g/g) and the mSBM diet (.42 g/g). The true ileal digestibilities of CP for the SC and SBM diets were about 16 and 12 percentage units higher, respectively, than for the mSBM diet ($P < .05$). The dietary TIA levels were positively correlated with ileal recoveries of total N, endogenous N, and dietary N ($r = .96$, $P < .01$; $r = .84$, $P < .01$, and $r = .94$, $P < .01$, respectively).

Table 7. Flow of DM and recoveries of total nitrogen (N), endogenous N, and dietary N in addition to the true digestibility of CP at the terminal ileum of pigs as affected by differently processed rapeseed products[a] as a sole protein source in the cornstarch-based diets (Trial II)

	Diet (g NDF/kg DM of diet)			
Item	RC1 (92)	RC2 (127)	RC3 (87)	RMSE[b]
No. of observations	6	6	3	-
Ileal DM flow, g/kg of DMI	193.9[d]	253.9[c]	195.0[d]	7.66
Nitrogen in ileal digesta				
Total N, g/kg of DMI	5.95[d]	8.10[c]	5.81[d]	.58
Endogenous, g/kg of DMI	2.24[c]	3.03[c]	2.89[c]	.57
Dietary N, g/kg of DMI	3.71[d]	5.07[c]	2.92[e]	.34
Endogenous N, g/g of total N	.38[d]	.37[d]	.49[c]	.05
True ileal CP digestibility, %	85.1[d]	80.9[e]	87.8[c]	1.34

[a] SC = soybean concentrate; SBM = toasted soybean meal; mSBM = mixture of toasted and untoasted soybean meal; RC1 = dehulled-toasted rapeseed cake; RC2 = non-dehulled-toasted rapeseed cake; RC3 = dehulled-untoasted rapeseed cake.

[b] Root of mean square error (df = 14).

[c,d,e] Means within a row lacking a common superscript letter differ ($P < .05$).

Trial II. The daily flow of ileal DM for the diets containing dehulled rapeseed cakes (RC1 and RC3) were lower than for the RC2 diet (non-dehulled-toasted; Table 7). The RC1 and RC3 diets had lower ileal losses of total N compared with the RC2 diet ($P < .05$). This difference resulted mostly from the lowered ileal losses of dietary N ($P < .05$). Compared with the RC2, the RC1 diet greatly decreased ENL ($P = .08$). The ratio of endogenous N to total N in ileal digesta of pigs for the RC3 diet was higher ($P < .05$) than for the RC1 or RC2 diets. The true ileal CP digestibility was increased ($P < .05$) by dehulling (RC2 diet vs RC1 diet), and it was decreased ($P < .05$) following toasting (RC3 diet vs RC1 diet). The dietary contents of NDF were positively correlated with ileal flow of DM and recoveries of total N and dietary N ($r = .97$, $P < .01$, $r = .91$, $P < .01$, and $r = .90$, $P < .01$, respectively).

Discussion

The purpose of this study was to determine the apparent ileal digestibility of protein and AA in diets containing various soybean and rapeseed products used as the sole protein sources. Another purpose was to measure ^{15}N-enrichment of N in ileal digesta and from that to estimate losses of endogenous N using the ^{15}N-isotope dilution method. This method involves labeling of the endogenous N by a continuous infusion of ^{15}N. The greatest advantage of using [^{15}N]leucine as a label is that leucine is a stable amino acid that has no important metabolic function other than for protein synthesis (Moughan et al., 1992).

The ^{15}N-isotope dilution method is based on the assumptions that during the collection period a steady state is achieved and ^{15}N-enrichment of the endogenous N in ileal digesta is similar to that in the TCA-soluble fraction of blood plasma. The first assumption has been questioned because of a possible rapid recycling of dietary AA and a diurnal variation in ^{15}N-enrichment excess in the TCA-soluble fraction of total N in blood plasma (de Lange et al., 1992). However, Schulze (1994) and Moughan et al. (1992) showed that a steady state condition could be reached between 5 and 8 d of continuous ^{15}N infusion. In the present study, the collection period started on d 7 of infusion (Figure 1).

The validity of the method has been mostly criticized with regards to the choice of precursor pool (Moughan et al., 1992). Lien et al. (1997a,b) showed that frequent blood sampling between feeding times gave good estimates for the ^{15}N-enrichment in endogenous N of blood plasma, in contrast to blood sampling at the time of feeding (de

Lange et al., 1990; Souffrant et al., 1993). The latter may lead to an underestimation of endogenous N recoveries in ileal digesta. Schulze (1994) sampled blood once at the midpoint between the 6-h feeding periods. It was further found that values obtained in studies by Schulze (1994) were similar to the mean value of three blood samplings between the feedings (our unpublished observations). In the present study, therefore, blood was sampled once at the midpoint between the 12-h feeding periods.

The direct incorporation of dietary AA into synthesis of intestinal protein (Alpers, 1972) and(or) transamination of AA in the gut wall, or their use by microbes may underestimate ENL (de Lange et al., 1992; Lien et al., 1997a,b). The variability of the contribution of urea and ammonia to total N in the TCA-soluble blood plasma upon feeding different diets may also contribute to the source of error when estimating ENL in digesta (de Lange et al., 1992; Lien et al., 1997b).

Nevertheless, at present, the ^{15}N-isotope dilution method is commonly used to determine values for ENL, and more especially, the relative differences in ENL in pigs associated with different dietary treatments (de Lange et al., 1990; Huisman et al., 1992; Krawielitzki et al., 1994; Schulze et al., 1994, 1995).

Composition of Diets

Diets were formulated to provide similar amounts of ID CP and first-limiting AA to ensure comparable levels of the dietary inclusion of feedstuffs in the following N balance study. The AID coefficients for CP determined for diets in the present study (Table 4) were similar or close to those obtained in a preliminary study (data unpublished). It was assumed that differences in the dietary CP contents did not influence apparent and true ileal digestibilities of CP (Donkoh and Moughan, 1994) and the AID of AA (Fan et al., 1994; van Barneveld et al., 1994).

Diets were designed to vary in dietary factors such as antinutritional factors (ANF; Trial I) or fiber content (NDF; Trail II). In soybeans, trypsin inhibitors (TI) and lectins are the major ANF leading to reduced nutritional value for farm animals (Huisman and Jansman, 1991). Trypsin inhibitor activity differed markedly among soybean products (Table 1). Thus, the TIA in the SC, SBM, and mSBM diets differed correspondingly (Table 3). The soybean diets also differed in their crude fiber content, mainly because of different amounts of added purified cellulose. It was assumed that purified cellulose would not influence the AID of CP and AA of the soybean diets (Fernández and

Jørgensen, 1986; Sauer et al., 1991). Moreover, Furuya and Kaji (1992) showed that purified cellulose does have a minor effect on ENL.

In a canola type rapeseed, fiber with its physical and chemical properties is a main constituent that affects the nutritional value of rapeseed products for nonruminant animals (Mitaru et al., 1984; Bjergegaard et al., 1991). In this respect, it seems that dehulling could be beneficial for the nutritional value of rapeseed. In Trial II, the rapeseed diets were formulated to contain different levels of NDF by using a non-dehulled rapeseed cake (RC2) in comparison with dehulled cakes (RC1, RC3). Of ANF in canola meal, phytates are the most important (Bell, 1984), because tannins and sinapine seem to be of minor nutritional significance (Blair and Reichert, 1984).

Heat-treatment during processing can decrease the nutritional value of protein in canola meal for animals (Anderson-Hafermann et al., 1993; Grala et al., 1994a,b). These studies showed that total content and digestibility of lysine can be decreased by excessive heating (higher than 100°C and longer than 20 min). In the present study, temperature treatment of 80 to 100°C during toasting aimed to improve the nutritional value of dehulled rapeseed cake by avoiding protein denaturation and reducing the glucosinolate content of RC1. Grala et al. (1994a,b) showed that mild heat-treatment (temperature up to 100°C) increased the nutritional value of canola meal. As a result of the mild toasting in the present study, the contents of AA, particularly lysine, stayed unchanged, and the glucosinolate level in RC1 was reduced by 30% compared with RC3 (Table 1).

Ileal Digestibilities of Crude Protein, Amino Acids, and Endogenous Nitrogen Losses Caused by Soybean Protein Sources

A high intake of TI limits proteolysis of dietary protein by the formation of inactive complexes with the pancreatic enzymes trypsin and chymotrypsin (Liener and Kakade, 1980). This may lead to compensatory secretions of endogenous proteins by the pancreas (Fukuoka et al., 1986) and an increased ENL. The result is a decreased apparent ileal protein digestibility. Moreover, due to inactivation of proteolytic enzymes and incomplete compensation by extra secretion, dietary protein may be digested to a lesser extent.

The results on the AID of CP were within the range of previously published data for a soy concentrate as a protein source (Peiniau et al., 1996). The AID of CP in the SBM diet were comparable to results for commercial SBM (de Lange et al., 1990; Sauer et al., 1991). Vandergrift et al. (1983) showed a decrease in the AID of CP and AA in pigs fed unheated soybean flakes compared with heated flakes (25 to 105 min at 100 to 107°C). The latter results, along with those of Herkelman et al. (1992), showed that heat-treatment of soybean, aimed to reduce TIA, was beneficial to the AID of CP and AA.

In the present study, the dietary level of TIA was negatively correlated with the AID of CP and the sum of AA of the soybean diets. Lower AID of CP were associated with higher ileal recoveries of endogenous and dietary N. The ileal recoveries of total and endogenous N were thus related to the dietary TIA levels. Studies of Schulze (1994), with a dose-response measurement of isolated soybean TI effect on ileal losses of endogenous and dietary N, corresponded with the present results. Moreover, similar to findings of Schulze (1994), an increase in ileal recovery of dietary N was much higher than that of endogenous N upon increased intakes of dietary TIA. The reduced true ileal CP digestibility of the mSBM diet compared with the SC or SBM diets ($P < .05$) indicates a possible inactivation of proteolytic enzymes by the high dietary TIA level.

The presence of lectins (Schulze, 1994) and(or) antigenic proteins (Lallès et al., 1993) could also contribute to the lower AID of nutrients in the mSBM diet that contained untoasted soybean meal. Combination of the present results (TIA levels and unknown contents of lectins and other ANF) with those of Schulze (1994), who used purified soybean TI and lectins, resulted in a lower apparent and true ileal digestibilities of CP in our study. The results of the literature cited above suggest, therefore, that effects of other ANF in soybean were also important in the present study.

Ileal Digestibilities of Crude Protein and Amino Acids, and Endogenous Nitrogen Losses Caused by Dehulling and Toasting of Rapeseed Cake

The high fiber content in rapeseed meal comes mainly from a hull content of about 25 to 30%. Rapeseed hulls contain about 60% NDF, of which lignin constitutes almost 50%. Of the carbohydrates present in the hulls, pectins, cellulose, hemicellulose, and β-glucans are the most important from the viewpoint of nutritional value for nonruminant animals, because these carbohydrates are not digested by intestinal enzymes (Bell, 1984). Moreover, these components may interact with the AA released during protein hydrolysis. This interaction allows some AA to avoid absorption in the small intestine and pass into the large intestine (Bergner et al., 1981; Howard et al., 1986). As a consequence, the above-mentioned constituents may decrease apparent and true ileal digestibilities of CP (Mosenthin et al., 1994; Bjergegaard et al., 1991, respectively), increase secretion and losses of intestinal mucin (Mariscal-Landin et al., 1995), and induce proliferation of epithelial and mucosal cells (Bjergegaard et al., 1991; Jin et al., 1994). Because these carbohydrates, especially pectins and hemicellulose, are highly fermentable, they may stimulate the growth of intestinal bacteria (Sauer et al., 1991; Jørgensen and Jensen, 1994; Mosenthin et al., 1994).

Dehulling. A considerable increase ($P < .05$) of the AID for DM, CP, and total AA resulted from dehulling (RC1 diet vs RC2 diet). A high negative relationship between the dietary NDF contents and these results were found. Mitaru et al. (1984) reported that fiber of rapeseed hulls decreases ileal digestibilities of all nutrients in pigs. Particularly, the lignin present in rapeseed hulls may have contributed to this negative effect (Blair and Reichert, 1984; Bjergegaard et al., 1991).

The present results were in agreement with studies of Schulze (1994), who measured the dose-response effect of purified NDF from wheat bran on the AID of CP and DM. However, the change in digestibility units per unit of extra purified NDF was lower than in the present study. This difference in the response to the dietary NDF contents between both studies suggests that other constituents of fiber in rapeseed hulls may also have affected the digestibilities of DM, CP, and AA in the present study. In contrast to the rapeseed hull fiber in the purified NDF, the negative impacts of such carbohydrates as pectins and β-glucans were unlikely to be present, because they were probably washed out during preparation. The variability in the fiber effects from different plant sources on the nutrient digestibilities should also be considered (Fernández and Jørgensen, 1986).

Compared with all other AA, threonine had the lowest AID in the RC2 diet. The low AID of threonine could result from an increased passage of mucus glycoproteins, which contain a large amount of threonine (Cetta et al., 1972). A direct indication for the lower threonine digestibility in the RC2, due to higher losses of endogenous protein, is a much higher ($P = .08$) loss of endogenous N for this diet than for the RC1 diet (differences of 35%). Low recoveries of endogenous N in ileal digesta were associated with low contents of NDF in the RC1 diet. This relationship agrees with Schulze et al. (1994, 1995), in their study of the dose-response effect of isolated NDF from wheat bran on the ENL of pigs. Comparison of results from the latter studies with the present results (NDF content and unknown contents of other organic matter constituents in hulls) shows a corresponding response of ENL to NDF in both studies. The ENL for the RC2 diet were similar to those estimated for canola type meal by Grala et al. (1997).

In the present study, recoveries of ileal dietary N were lower for the RC1 than for the RC2 diet ($P < .05$), and consequently resulted in a difference ($P < .05$) in the true ileal CP digestibility. Losses of ileal dietary N were negatively correlated with the NDF content of the diets. Bjergegaard et al. (1991) showed that the low true CP digestibility of canola meal is mainly caused by the large proportion of N enclosed in the fiber fraction (cell wall components), where enzymes have restricted access for digestion. The present studies, along with those of de Lange et al. (1990, 1992) and Grala et al. (1997), demonstrate that rapeseed fiber (NDF) considerably affects ileal losses of dietary and endogenous N in pigs. It can be concluded that dehulling of canola meal (cake) removes fiber constituents that cause increased ileal losses of endogenous and dietary N.

Toasting. Mild heat-treatment may increase the nutritional value of rapeseed protein (Rayner and Fox, 1976; Grala et al., 1994b). This positive effect may be explained by physical-chemical modifications of plant cells in the carbohydrate fraction. The cell contents become more accessible for digestive enzymes in the small intestine, resulting in a higher digestibility of nutrients. In the present studies, the AID of DM, CP, and AA were not affected by the mild toasting of dehulled rapeseed cake (RC3 diet vs RC1 diet). Thus, the removal of hulls together with the fiber fraction, to which the mild toasting would be more beneficial (Peisker, 1994), did not affect the nutritional value of dehulled rapeseed cake. However, an increased loss of dietary N and reduced true ileal CP digestibility of the RC1 diet (dehulled-toasted cake) compared with the RC3 diet (dehulled-untoasted cake) may suggest that significant denaturation of protein occurred in the toasted cake (Hurrell, 1990). On the other hand, there was a tendency toward decreased ENL in pigs fed the RC1 diet (difference of 29% compared with the RC3 diet). This decrease in ENL may suggest that toasting of dehulled rapeseed cake was beneficial in terms of reducing the remaining effects of myrosinase and harmful products from the degradation of glucosinolates still present in untoasted canola meal (Grala et al., 1994b).

Implications

The present study showed that apparent ileal digestibilities of protein and amino acids in pigs were influenced adversely by high ileal losses of endogenous and dietary nitrogen. A high trypsin inhibitor activity in the soybean products fed to pigs is associated with high ileal losses of endogenous and dietary nitrogen. Dehulling increases apparent ileal digestibilities of protein and amino acids and the true ileal protein digestibility in pigs fed diets with rapeseed cake. Rapeseed hulls, predominantly their fiber, increase endogenous and dietary nitrogen in ileal digesta of pigs. Toasting of dehulled rapeseed cake does not affect the apparent ileal digestibility of protein and amino acids while it decreases the true ileal protein digestibility.

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CHAPTER 3

NITROGEN UTILIZATION IN PIGS FED DIETS WITH SOYBEAN AND RAPESEED PRODUCTS LEADING TO DIFFERENT ILEAL ENDOGENOUS NITROGEN LOSSES

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Nitrogen Utilization in Pigs Fed Diets with Soybean and Rapeseed Products Leading to Different Ileal Endogenous Nitrogen Losses

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ABSTRACT

Nitrogen (N) balance was determined in 36 pigs (BW 24 to 30 kg) fed diets inducing different ileal endogenous N losses (ENL). We tested the hypothesis that enhanced ENL may be indicative of a higher recycling of endogenous proteins that will induce a greater urinary N loss and a lower efficiency of the dietary N utilization for retention. The cornstarch-based diets contained either soy concentrate (SC), soybean meal (SBM), a mixture of toasted and untoasted soybean meal (mSBM), dehulled-toasted rapeseed cake (RC1), non-dehulled-toasted rapeseed cake (RC2), or dehulled-untoasted rapeseed cake (RC3). The diets were balanced for their content of apparent ileal digestible (ID) CP (108 g/kg feed) and apparent ID of Lys, Thr, Met+Cys, Trp, and Ile. Feeding level was 2.7 times ME for maintenance per kilogram $BW^{.75}$ and restricted to 88% of the requirements for ID Lys as the first-limiting amino acid. During a 5-d period, urine and feces were collected daily in metabolism cages. Compared with the SC diet (low ENL), the diets with SBM (medium ENL) and mSBM (high ENL) resulted in a greater ($P < .05$) urinary N excretion. Nitrogen retention tended to be less ($P = .08$) in pigs fed diets that caused greater ENL. The utilization of ID N for retention in pigs fed the mSBM diet was lower ($P < .05$) than for those fed the SC diet. There were no differences in urinary N excretion, N retention, and the utilization of ID N for retention in pigs fed the rapeseed diets of different fiber contents (hulls as the NDF source). We concluded that, at similar intakes of the first-limiting amino acid, N retention in pigs fed soybeans tended to be reduced by greater ENL as induced by antinutritional factors (e.g., trypsin inhibitors). Rapeseed hulls, as the predominant fiber source, do not affect N retention and the utilization of ID N for retention.

Key Words: Nitrogen Balance; Soybeans; Rapeseed; Pigs

Introduction

Secretion and(or) absorption of endogenous N is influenced by a number of animal and dietary factors (Nyachoti et al., 1997). Souffrant et al. (1993) and Krawielitzki et al. (1994, 1996) showed that approximately 25% of the total endogenous protein secretions are present in the ileal digesta of pigs. When endogenous N losses (**ENL**) result substantial losses of limiting amino acids (**AA**), the AA and energy requirements for maintenance are also increased (de Lange et al., 1995). The great demand for replenishment of ENL will be at the expense of AA for growth (Fuller, 1991; Moughan, 1993). Consequently, less AA will be used for N retention, resulting in an excessive supply of other AA. These AA will be catabolized, and N excreted in urine. Because the pattern of AA required for maintenance differs from that required for body gain, it will also lead to AA oxidation, resulting in an additional urinary N excretion (Moughan, 1995;

Sève and Henry, 1996). Therefore, our hypothesis was that the level of recycled endogenous AA may have negative consequences for the efficiency of dietary AA utilization for N retention in pigs. In this respect, Grala et al. (1997) showed that high ENL caused by peas or canola meal were associated with greater urinary N losses and less utilization of dietary N for retention compared with wheat gluten that had low ENL. The objective of this study was to investigate the effects of dietary factors in differently processed soybean and rapeseed products, inducing various ENL, on N balance in growing pigs.

Materials and Methods

Protein Sources. Three soybean and three rapeseed products were used in two separate trials. In Trial I, the soybean products were commercial soybean concentrate (**SC**), dehulled-toasted soybean meal (**SBM**), and dehulled-untoasted soybean meal (**uSBM**). The SC, SBM, and uSBM were prepared from different batches of soybeans.

In Trial II, the rapeseed cake products were prepared by CETIOM-GERDOC (Pessac, France) from one batch of rape oilseeds (*Brassica napus* L.) of a low glucosinolate variety (canola type). The rapeseed cakes were as follows: dehulled-toasted rapeseed cake (**RC1**), non-dehulled-toasted rapeseed cake (**RC2**), and dehulled-untoasted rapeseed cake (**RC3**). A more detailed description of the technological processing of the soybean and rapeseed products and their chemical characteristics have already been presented by Grala et al. (1998).

Animals and Housing. Thirty-six 11- to 12-wk-old Polish Landrace barrows with a BW (± SE) of 21.3 kg (± .3 kg) upon arrival were used in the study. The pigs were individually housed in metabolism cages at an ambient room temperature of 22 to 24°C and relative air humidity of approximatelly 55%. Cages were made of galvanized steel and allowed the collection of urine via funnels situated underneath them. During a 10-d adaptation period, pigs were fed a commercial diet. After this period, pigs were randomly allocated to six dietary treatments (six observations per treatment), and the experimental diets were introduced.

Diets and Feeding. The ingredient composition of diets is presented in Table 1. During diet preparation, SBM and uSBM were mixed in a proportion of 75:25, and the diet was coded as **mSBM diet**. The other soybean diets were coded as **SC diet** and **SBM diet**, and the rapeseed diets as **RC1 diet, RC2 diet, and RC3 diet**. The soybean and rapeseed products were the only protein sources in the cornstarch-based diets. The

Table 1. Composition of the experimental diets[a] (%)

Item	SC diet	SBM diet	mSBM diet	RC1 diet	RC2 diet	RC3 diet
Soy concentrate	17.85	-	-	-	-	-
Soybean meal	-	27.75	24.36	-	-	-
Untoasted soybean meal (uSBM)	-	-	8.12	-	-	-
Dehulled-toasted rapeseed cake	-	-	-	37.00	-	-
Non-dehulled-toasted rapeseed cake	-	-	-	-	44.50	-
Dehulled-untoasted rapeseed cake	-	-	-	-	-	38.30
Cornstarch	52.08	45.98	43.39	42.09	33.57	41.40
Dextrose	15.00	15.00	15.00	15.00	15.00	15.00
Soy oil	1.30	1.30	1.30	-	2.30	-
Cellulose	8.00	5.10	3.20	.75		.30
$CaCO_3$	1.16	1.20	1.20	.88	.77	.90
Monocalcium phosphate	1.80	1.70	1.65	1.50	1.35	1.50
MgO	.10	.05	-	-	-	-
NaCl	.30	.30	.30	.30	.30	.30
$KHCO_3$	.75	.10	-	.75	.45	.75
$NaHCO_3$	.25	.25	.25	.25	.25	.25
Mineral-vitamin mixture[b]	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine	.11	.04	-	.33	.35	.26
L-Threonine	.12	.07	.08	.05	.04	.02
DL-Methionine	.14	.14	.13	.05	.05	-
L-Cystine	.02	-	-	-	.02	-
L-Isoleucine	-	-	-	.03	.03	-
L-Tryptophan	.02	.02	.02	.02	.02	.02

[a] SC = soy concentrate; SBM = toasted soybean meal; mSBM = mixture of toasted and untoasted soybean meal; RC1 = dehulled-toasted rapeseed cake; RC2 = non-dehulled-toasted rapeseed cake; RC3 = dehulled-untoasted rapeseed cake.

[b] The premix supplied per kilogram of feed: 9,000 IU of vitamin A; 1,800 IU of vitamin D_3; 40 mg of vitamin E; 5 mg of riboflavin; 30 mg niacin; 12 mg of d-pantothenic acid; 1,000 mg of choline, 40 µg of vitamin B_{12}; 2 mg of thiamine; 3 mg of pyridoxin; .1 mg of biotin; 1 mg of folic acid; 3 mg of vitamin K; 50 mg of ascorbic acid; 72.8 mg of Zn ($ZnSO_4{\cdot}H_2O$); 44 mg of Mn (MnO_2); 80 mg of Fe ($FeSO_4{\cdot}7H_2O$); .525 mg of Co ($CoSO_4{\cdot}5H_2O$); .38 mg of K (KI); .254 mg of Cu ($CuSO_4{\cdot}5H_2O$); .06 mg of Se ($Na_2SeO_3{\cdot}5H_2O$); 40 mg tylosin.

diets were balanced for the content of apparent ileal digestible (ID) CP (N × 6.25) of 108 g/kg diet and apparent ID Lys, Met, Met+Cys, Thr, Trp, and Ile (88% of the requirements for ID Lys for pigs of 25 to 45 kg BW; CVB, 1994). The pigs used in the present study were of similar genetic background and growth potential as those, on which the Dutch feeding standards are based. Apparent ileal digestibilities (AID) of CP and AA, and ENL were determined for similar diets in a previous study (Grala et al., 1998). Analyzed and calculated chemical compositions of the experimental diets are presented in Table 2.

Pigs were fed twice daily in equal meals at 0800 and 1600 at the level of 2.7 times the maintenance requirement for energy (2.7×420 kJ ME/kg$^{.75}$; ARC, 1981). The ME was estimated from its content in the ingredients of the diets (CVB, 1994). The mash feed was mixed with water (1:2, wt/vol) just before feeding. No additional water was supplied after the feeding.

Experimental Procedure. The N balance trial consisted of a 7-d adaptation period followed by a 5-d collection period. Feces were collected in plastic bags attached to the pigs (Van Kleef et al., 1994), which were changed at least twice daily, and stored frozen (−20°C) pending analysis. Urine was collected in plastic containers via the funnels underneath the cages. The funnels were frequently washed with acidified water during the day. Urine was removed once each day and stored at 4°C pending analysis. To prevent N losses during collection and storage, 8 mL of 25% H_2SO_4 was added to each container every day. The average BW of barrows at the beginning of the N balance period was 26.5 kg (± .4 kg), and at the end of the trials the average BW was 29.7 kg (± .5 kg).

Nitrogen intake and fecal and urinary N excretion were measured during the N balance period. Nitrogen retention was calculated as N intake minus the sum of fecal and urinary N. The apparent fecal digestibility of CP (N × 6.25) and DM in the experimental diets was determined using chromic oxide (Cr_2O_3) as an indigestible marker (1.0 g/kg as-fed diet). To calculate the digestibility coefficients of dietary CP, the N in the diets was corrected for N added as crystalline AA, assuming that there was 100% absorption from the ileum. The coefficients for fecal CP digestibilities were used to calculate the urinary N excretion corrected for N absorbed from the large intestine. The correction for urinary N excretion was estimated as the difference between the fecal and ileal CP apparent digestibilities, based on the assumption that all N resulting from any protein fermentation will be absorbed from the large intestine and excreted in urine. The calculations were made according to the formula $NU_c = NI \times N_d / N_{dAA} \times (DCN_f - DCN_i) / 100$, where NU_c is corrected urinary N, NI is N intake, N_d is dietary N, N_{dAA} is

Table 2. Chemical composition of the experimental diets[a] (g/kg as-fed)

Item	SC	SBM	mSBM	RC1	RC2	RC3
Chemical composition (analyzed)						
DM	895.5	896.4	895.7	906.6	900.2	905.4
CP (N × 6.25)	116.3	129.4	149.4	131.3	145.0	128.8
Ether extract	17.4	17.7	17.4	69.5	70.3	72.2
Crude fibre	57.0	45.7	33.8	45.5	56.1	44.9
Ash	47.7	46.8	47.0	54.0	52.1	52.7
NDF	ND[b]	ND	ND	81.4	113.9	78.3
TIA, mg/g[c]	.40	1.37	3.38	ND	ND	ND
Amino acids (calculated)[d]						
Lysine	7.9	8.7	9.5	8.8	9.7	8.6
Methionine	3.0	3.1	3.4	2.8	3.0	2.8
Methionine+cystine	4.8	5.3	5.8	5.7	6.3	5.7
Threonine	5.3	5.8	6.6	6.2	7.1	6.0
Isoleucine	5.1	5.8	7.2	5.5	6.1	5.5
Tryptophan	1.6	1.8	2.1	1.9	2.0	1.8
Apparent ileal digestible (ID)-CP and amino acids (calculated)[e]						
ID CP (N × 6.25)	101.1	107.5	101.8	100.4	101.0	98.0
ID lysine	7.4	7.9	7.6	7.0	7.4	7.0
ID methionine	2.7	2.8	2.6	2.5	2.6	2.5
ID Met+Cys	4.1	4.4	4.3	4.7	5.1	4.7
ID threonine	4.3	4.6	4.4	4.7	5.0	4.5
ID isoleucine	4.7	5.0	4.9	4.4	4.6	4.4
ID tryptophan	1.4	1.5	1.4	1.5	1.6	1.5
ME, MJ/kg[f]	14.4	14.4	14.4	14.4	14.4	14.4

[a] SC = soy concentrate; SBM = toasted soybean meal; mSBM = mixture of toasted and untoasted soybean meal; RC1 = dehulled-toasted rapeseed cake; RC2 = non-dehulled-toasted rapeseed cake; RC3 = dehulled-untoasted rapeseed cake.

[b] Not determined.

[c] Trypsin inhibitor activity (TIA) expressed in milligrams of trypsin inhibited per gram of diet; calculated based on the analyzed TIA of the protein sources.

[d] Based on the analyzed amino acid (AA) content of the protein sources, corrected for the analyzed CP content of the diets.

[e] Based on the analyzed CP and calculated AA contents of the diets (footnote d), and then corrected for the apparent ileal digestibility coefficients (Grala et al., 1998).

[f] ME was calculated from its content in the ingredients (CVB, 1994).

dietary N corrected for N of crystalline AA, DCN_f is apparent fecal CP digestibility of the diet (Tables 3 and 5), and DCN_i is the AID of CP of the diet (Grala et al., 1998). The efficiencies of ID N utilization for N retention were also calculated, using the coefficients for the AID of CP, including the N from crystalline AA. In Trials I and II, N intake, fecal and urinary N, and N retention were calculated per kilogram of $BW^{.75}$ to correct for the variation in N intake, resulting from differences in BW of pigs at the start of the N balance period.

Sample Preparation and Analysis. Samples of feces and urine were pooled within the 5-d N balance period for each pig. Fresh samples of feces were taken for analysis of DM and N (AOAC, 1984). A subsample of feces was freeze-dried for analysis of Cr_2O_3 (Bosch et al., 1988). The same procedures were used for analyses of DM, N, and Cr_2O_3 in the diets. Diets were also analyzed for ether extract (ISO 6492, 1996), crude fiber (NEN 5417, 1988), and NDF (Englyst and Cummings, 1988). Amino acids were analyzed as described by Andrews and Baldar (1985). The soybean products were analyzed for trypsin inhibitor activity (TIA) as described by Van Oort et al. (1989). Glucosinolates in rapeseed products were determined according to the method 1864/90 (EEC, 1990).

Statistical analysis was performed using the GLM procedure of SAS (1990). The effect of dietary treatment within each trial was tested against the residual error according to the model $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} is the dependent variable, μ is overall mean, T_i is treatment (within each trial: i = 1, 2, 3), and e_{ij} is residual error. The differences among treatments were determined for the leastsquare means (LSM) using multiple comparisons of difference (PDIFF) with adjustment for the Tukey-Kramer test

Table 3. Apparent fecal digestibilities of the cornstarch-based diets supplemented with soy concentrate (SC), soybean meal (SBM), or a mixture of toasted and untoasted soybean meal (mSBM[a]) fed to growing pigs (Trial I)

Item	Diet			
	SC	SBM	mSBM	RMSE[b]
DM	96.6	96.5	96.4	.68
CP[c]	87.8[d]	85.8[d]	76.7[e]	1.86

[a] Mixture of toasted and untoasted soybean meal in a ratio of 75:25, respectively.

[b] Root of mean square error (df = 12).

[c] Values corrected for N added to the diets as crystalline amino acids, assuming their intestinal absorption of 100%.

[d,e] Means within a row lacking a common superscript letter differ ($P < .05$).

to detect for significance at $P < .05$ (SAS, 1990). In each treatment of the SC, SBM, mSBM, RC1, and RC2 diets one pig had to be excluded from the experiment because of large feed refusals. Data from these pigs were treated as missing values.

Results

Trial I. The contents of CP in the diets ranged from 116.3 to 149.4 g/kg (as-fed basis), and were somewhat lower than those calculated from the CP content of ingredients (Table 2). As a consequence of these differences in the dietary CP contents, the calculated contents of apparent ID CP and first-limiting AA for the SBM diet seemed to be higher than those for the SC and mSBM diets. The ID CP contents for the SC and mSBM diets were about 6% lower than for the SBM diet. However, the ratio of the first-limiting AA to ID Lys was unchanged for each diet. Based on that ratio, the potential for N retention of pigs fed the SC diet was 3 and 7% lower than for those fed the mSBM and SBM diets, respectively.

The mSBM diet had a lower fecal CP digestibility than the SC and SBM diets ($P < .05$), and the digestibility of DM did not differ among diets ($P > .05$; Table 3). Results for partitioning of dietary N in pigs are presented in Table 4. Nitrogen intake differed among treatments ($P < .05$) and corresponded to the CP contents of the diets. Nitrogen excretion in feces for the mSBM diet was greater than for the SBM ($P < .05$), which was greater than for the SC diet ($P <. 05$). Urinary N excretion for the SBM diet and the mSBM diet were more than for the SC diet ($P < .05$). A correction made for N in urine absorbed from the large intestine did not eliminate differences in urinary N excretion. The corrected urinary N excretions for pigs fed the SC diet were less ($P < .05$) and tended to be less ($P = .07$) than for those fed the SBM diet and mSBM diets, respectively. Nitrogen retention in pigs tended to differ among treatments ($P = .08$). The value for the SC diet tended to be greater than that for the mSBM diet ($P = .07$). Nitrogen retention (in percentage of N intake) was markedly more for the SC diet than for the SBM diet and mSBM diet ($P < .05$). The utilization of ID N for N retention in pigs fed the SC diet was 11 and 18% greater than for those fed the SBM diet ($P > .05$) and the mSBM diet ($P < .05$), respectively.

Trial II. The contents of CP in the diets ranged from 128.8 to 145.0 g/kg (as-fed basis) and were slightly lower than those calculated from the CP content in ingredients (Table 2). The difference between planned and analyzed contents of CP resulted in a slightly lower (by 3%) content of apparent ID CP for the RC3 diet than for the RC1 and

RC2 diets. The ratio of the first-limiting AA to ID Lys in each diet stayed unchanged, however, compared with the intended values. Based on that ratio, the potential for N retention in pigs fed the RC2 diet was about 5% greater than for those fed the RC1 and RC3 diets.

The RC2 diet had a lower ($P < .05$) apparent fecal digestibility for DM and CP than the RC1 and RC3 diets (Table 5). The DM and CP digestibilities for the RC1 and RC3 diets were not different from those for the RC2 diet, so it seems that the digestibilities were affected by dehulling, but not by toasting.

Results for dietary N partitioning in pigs are presented in Table 6. Pigs fed the RC2 diet had a greater N intake and N excretion in feces than those fed the other two diets ($P < .05$). There were no differences in urinary N excretion (total and corrected for

Table 4. Nitrogen (N) partition in pigs receiving semisynthetic diets balanced for ileal digestible protein and amino acids, containing soy concentrate (SC), soybean meal (SBM), or a mixture of toasted SBM and untoasted soybean meal (mSBM[a]), which caused different ileal endogenous N losses (Trial I)

Item	Diet			
	SC	SBM	mSBM	RMSE[b]
Intake, $g/(kg^{.75})\cdot d^{-1}$	1.480[f]	1.630[g]	1.893[h]	.080
Fecal output, $g/(kg^{.75})\cdot d^{-1}$	.175[f]	.228[g]	.436[h]	.026
Ileal digestible, $g/(kg^{.75})\cdot d^{-1}$ [c]	1.283[f]	1.351[f]	1.289[f]	.063
Urinary output, $g/(kg^{.75})\cdot d^{-1}$	.385[f]	.531[g]	.675[h]	.071
Urinary N absorbed from LI, $g/(kg^{.75})\cdot d^{-1}$ [d]	.016	.047	.161	ND
Urinary N corrected, $g/(kg^{.75})\cdot d^{-1}$ [e]	.369[f]	.484[fg]	.514[g]	.071
Retention, $g/(kg^{.75})\cdot d^{-1}$	.920[f]	.871[f]	.783[f]	.088
Retention/intake, %	62.2[f]	53.4[g]	41.4[h]	4.84
Retention/ileally digested, %	71.7[f]	64.4[fg]	60.8[g]	5.92

[a] Mixture of toasted and untoasted soybean meal in a ratio of 75:25, respectively.

[b] Root of mean square error (df = 12). ND = not determined.

[c] Ileal digestible N as calculated (N intake × Digestibility coefficient of N [Grala et al., 1998] / 100].

[d] Urinary N absorbed from the large intestine (LI) is based on the difference between fecal (Table 3) and ileal (Grala et al., 1998) apparent CP digestibilities of the diets in relation to N intake.

[e] Corrected urinary N equals urinary N output minus urinary N absorbed from LI (see footnote d).

[f,g,h] Means within a row lacking a common superscript letter differ ($P < .05$).

Table 5. Apparent fecal digestibility (%) of the cornstarch-based diets supplemented with dehulled-toasted rapeseed cake (RC1), non-dehulled-toasted rapeseed cake (RC2), or dehulled-untoasted rapeseed cake (RC3) fed to growing pigs (Trial II)

Item	Diet			
	RC1	RC2	RC3	RMSE[a]
DM	96.2[d]	93.7[c]	96.0[d]	.42
CP[b]	82.6[d]	75.8[c]	81.0[d]	2.30

[a] Root of mean square error (df = 13).

[b] Values corrected for N added to the diets as crystalline amino acids, assuming their intestinal absorption of 100%.

[c,d] Means within a row lacking a common superscript letter differ ($P < .05$).

N absorbed from the large intestine) and in N retention in pigs ($P > .05$). Also, N retention as a percentage of N intake and the utilization of ID N for retention did not differ among treatments ($P > .05$).

Discussion

Only amino acids that are absorbed before the end of the small intestine are available for protein synthesis in an animal (Żebrowska et al., 1978; Just et al., 1981). For maximal AA utilization for protein deposition, an optimal pattern of essential AA is needed (Fuller et al., 1989; Wang and Fuller, 1989), and the energy supply must not be limiting in the diet (Bikker, 1994).

Dietary factors, such as antinutritional factors (**ANF**) and(or) fiber, may influence secretion and excretion of endogenous gut protein (Schulze, 1994). It should be recognized that only a fraction of the protein secreted into the gastrointestinal tract is recovered at the terminal ileum as ENL (Souffrant et al., 1993; Krawielitzki et al., 1994; 1996). The relationship between the amount of endogenous protein secreted into the gut and ENL will most likely vary with the type of diet (Schulze, 1994; Nyachoti et al., 1997). Consequently, the metabolic costs associated with ENL are likely to vary with the diet (de Lange et al., 1995). Therefore, the great demand for AA for maintenance processes (e.g., protein synthesis in the gut and other visceral organs) may contribute to the inefficiency of utilizing dietary N (AA) for body protein retention (Nyachoti et al., 1997). Fuller (1991) and Moughan (1993) showed that maintenance processes have a priority over growth processes. The recycling of endogenous protein associated with

Table 6. Nitrogen (N) partition in pigs receiving semi-synthetic diets balanced for ileal digestible protein and amino acids, containing dehulled-toasted rapeseed cake (RC1), non-dehulled-toasted rapeseed cake (RC2), or dehulled-untoasted rapeseed cake (RC3), which caused different ileal endogenous N losses (Trial II)

Item	Diet			
	RC1	RC2	RC3	RMSE[a]
Intake, g/(kg$^{.75}$)·d^{-1}	1.687^{ef}	1.825^{e}	1.649^{f}	.110
Fecal output, g/(kg$^{.75}$)·d^{-1}	.284^{f}	.427^{e}	.307^{f}	.040
Ileal digestible, g/(kg$^{.75}$)·d^{-1} [b]	1.286^{e}	1.266^{e}	1.252^{e}	.081
Urinary output, g/(kg$^{.75}$)·d^{-1}	.555^{e}	.544^{e}	.546^{e}	.086
Urinary N absorbed from LI, g/(kg$^{.75}$)·d^{-1} [c]	.104	.113	.082	ND
Urinary N corrected, g/(kg$^{.75}$)·d^{-1} [d]	.450^{e}	.431^{e}	.464^{e}	.083
Retained, g/(kg$^{.75}$)·d^{-1}	.848^{e}	.854^{e}	.796^{e}	.100
Retention/intake, %	50.3^{e}	46.8^{e}	48.2^{e}	4.77
Retention/ileally digested, %	66.1^{e}	67.4^{e}	63.5^{e}	6.37

[a] Root of mean square error (df = 13). ND = not determined.

[b] Ileal digestible N as calculated [N intake × Digestibility coefficient of N [Grala et al., 1998] / 100].

[c] *Urinary N absorbed from the large intestine (LI) is based on the difference between fecal (Table 5) and* ileal (Grala et al., 1998) apparent CP digestibilities of the diets in relation to N intake.

[d] *Corrected urinary N equals urinary N output minus urinary N absorbed from LI (see footnote c).*

[e,f,g] Means within a row lacking a common superscript letter differ (*P* < .05).

ENL will contribute to the AA catabolism and result in urinary N losses (de Lange et al., 1995; Moughan, 1995). Enhanced use of the dietary first-limiting AA for maintenance purposes, including AA that can be drained directly from the intestinal lumen for the synthesis of endogenous gut protein (Hirschfield and Kern, 1969; Alpers, 1972), will lead to an imbalance in the dietary AA pattern. As a result, the oxidation of AA that are then supplied in excess will cause additional urinary N losses (Benevenga et al., 1993; Sève and Henry, 1996). We hypothesized, therefore, that more ENL are indicative of more recycling of endogenous proteins that will induce a higher urinary N loss and decrease the efficiency of the dietary N utilization for retention.

It should be noted that heat treatment, involved in the processing of such feeds as soybean and canola meal, may have a negative effect on the biological availability of AA but have only a small effect on their apparent ileal digestibilities (Batterham, 1992). The products of Maillard reaction that may form during heat processing (e.g., complexes of AA with sugars, fats, and polyphenols) are largely absorbed by the intestine, but they are metabolized and excreted in urine (Finot, 1990). Depending on the extent of formation of those complexes, the excretion of their metabolic end-products in urine may add to N losses and inefficiency of dietary N utilization. However, the processing conditions used to process the soybean and rapeseed products were within a range that does not affect protein quality (Hancock et al., 1990; Grala et al., 1994). In this respect, the AA availabilities of protein sources used in the present study were not likely to have been affected by the processing.

The main objective of this investigation was to study the relationship between different losses of ileal endogenous N, induced by differently processed soybean and rapeseed products, on N balance in pigs fed diets containing similar levels of apparent ID CP and first-limiting AA. It was of special interest to investigate the consequence of ENL on the N partitioning of dietary N between utilization for retention and urinary losses. The relevance of the ^{15}N-isotope dilution technique for determination of ENL in pigs fed different type of diets has been reported previously (Grala et al., 1998).

Diet Composition

As intended, diets in the present study and in the preceding one (Grala et al., 1997a; unpublished data) had nearly the same composition. Therefore, it was assumed that in both investigations pigs would respond similarly to the dietary treatments in terms of nutrient digestibilities, as well as recycling of endogenous protein and ENL. Moreover, for Trial I, it was assumed that the greater ENL were related to a higher TIA level of the soybean-product diets. In Trial II, the greater ENL were primarily related to a higher fiber (NDF) content in the diet with non-dehulled-toasted rapeseed cake (RC2). However, it should be noted that other ANF in the soybean products and rapeseed hulls could also interfere with ENL, as reported previously (Grala et al., 1998).

The inaccuracies associated with diet preparation gave some differences in the ID CP and AA contents compared with the designed values. Nevertheless, the unchanged ratio of first-limiting AA to ID Lys (CVB, 1994) did not support additional urinary N losses, which could result from the extra oxidation of imbalanced AA. As outlined previously, because of more ID CP content in the SBM and mSBM diets compared with the SC diet, the N balance might be affected, resulting in more N retention in pigs fed these first two diets. Similarly, for the rapeseed-based diets, the RC2 diet, which contained slightly more ID CP, might give more N retention in pigs compared with the other two diets.

Effects of Soybean Products on Nitrogen Balance

Major differences in N intakes among treatments diminished when fecal N outputs were subtracted. When comparing pigs that ingested similar amounts of ID N, part of the higher urinary N excretion in pigs fed the mSBM diet can be explained by more N absorption from the large intestine compared with those fed the SC diet (Low and Żebrowska, 1989). The corrected urinary N excretion in pigs fed the mSBM diet was markedly greater than in pigs fed the SC diet, despite a similar ratio of first-limiting AA to ID Lys in both diets. However, there was a tendency for reduced N retention in pigs fed the mSBM diet compared with the SC diet. Moreover, the reduced efficiency of ID N utilization for retention in pigs fed the mSBM compared with the SC diet support the earlier outlined supposition that greater ENL could lead to the replenishment of the lost endogenous AA by dietary AA. Therefore, these results may support the preceding hypothesis that greater ENL induce more recycling of endogenous proteins that will lead to in a greater urinary N loss and higher inefficiency of the dietary N utilization for retention.

Effects of Rapeseed Products on Nitrogen Balance

An effect of dehulling (RC2 vs RC1), but not of toasting (RC1 vs RC3), on the ENL by pigs was found by Grala et al. (1998). The latter study showed that rapeseed hull fiber (NDF) was the predominant cause of a lower nutritional value for the RC2 diet. Therefore, the following discussion will address the effect of rapeseed fiber (NDF in hulls) on N balance in pigs. Markedly higher ENL in pigs fed the RC2 diet were not associated with greater urinary N excretion compared with those fed the RC1 diet. The corrected urinary N excretion and N retention were also not affected. The difference in the fecal N output between the RC2 diet and the RC1 or RC3 diets was not related to a shift in N excretion from the urine to the feces (Malmlöf and Håkansson, 1984; Schulze et al., 1993). Some studies have shown increases in N retention and the utilization of fecal digestible N when incremental additions of fiber provided an extra energy supply for pigs (Malmlöf and Håkansson, 1984; Stanogias and Pearce, 1985; Lenis et al., 1996). In Trial II, as outlined earlier, pigs fed the RC2 diet had somewhat greater potential for N retention than those fed the other two diets. However, based on the ingested amounts of ID CP, this difference diminished to great extent at least for the RC1 and RC2 diets. Neither the utilization of ingested ID N nor the utilization of ID N for retention differed among treatments. Therefore, these results illustrate the suggestion of Lenis et al. (1996) that fiber per se does not reduce the utilization of ID AA for N retention, meaning that hull fiber is the major antinutritive factor in rapeseed/canola meal (Bell, 1984).

The results of Trial II contradict the results of Trial I, in which ANF-dependent dietary components (e.g., TIA) caused greater ENL. It seems, therefore, that the hypothesis tested in this study cannot be confirmed with respect to the hull fiber and(or) other hull components of rapeseed products. It should be noted, however, that the primary objective of the study was not to determine the differences between the Trials I and II (e.g., the soybean and rapeseed diets differed in the levels of some first-limiting ID AA). In this respect, comparisons of the results of both trials should be interpreted with caution. Nevertheless, it seems that the ANF-dependent ENL have a greater impact on the metabolic costs for the pig (a significant impact on the ID N utilization for retention) than the fiber-dependent ENL (no effect on the ID N utilization for retention).

Implications

Extra loss of ileal endogenous nitrogen, induced by antinutritional factors, is associated with greater excretion of nitrogen in urine. This loss results in a tendency for a lower nitrogen retention and a lower utilization of dietary nitrogen for retention. Rapeseed hulls, as a predominant fiber source, do not affect nitrogen retention and the utilization of ileal digestible nitrogen for retention.

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CHAPTER 4

APPARENT PROTEIN DIGESTIBILITY AND RECOVERY OF ENDOGENOUS NITROGEN AT THE TERMINAL ILEUM OF PIGS FED DIETS CONTAINING VARIOUS SOYBEAN PRODUCTS, PEAS OR RAPESEED HULLS

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Apparent protein digestibility and recovery of endogenous nitrogen at the terminal ileum of pigs fed diets containing various soybean products, peas or rapeseed hulls

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ABSTRACT

Effects of various feed products, containing different dietary factors, on the apparent ileal digestibility of CP (N x 6.25) and on the recovery of ileal endogenous nitrogen (N) in piglets were investigated. Ileal endogenous N was measured using the ^{15}N-isotope dilution method. Thirteen piglets (BW of 12-23 kg) were fed maize starch-based diets containing either a soya concentrate (SC), purified rapeseed hulls (pRH) and SC (SRH diet), soybean meal (SBM), a mixture of toasted and untoasted soybean meal (mSBM) or peas. The apparent ileal digestibility (AID) of dry matter (DM) for the SC, SRH, SBM, mSBM and pea diets were 82.1, 74.3, 83.3, 80.0 and 74.9% ($P < 0.05$), respectively. The AID of CP for these diets were 82.4, 67.6, 81.6, 68.0 and 76.9% ($P < 0.05$), respectively. Similar differences in the AID for amino acids (AA) in those diets were found. The AID for CP and for the sum of AA in the pRH, as calculated by the difference between the SC and SRH diets, were 26% and 41%, respectively. For the SC, SRH, SBM, mSBM and pea diets, ileal recoveries (g/kg of DM intake) for endogenous N were 2.79, 3.46, 2.73, 4.89 and 3.29 ($P < 0.05$), respectively, and for dietary N were 1.16, 4.11, 1.64, 4.15 and 2.53 ($P < 0.05$), respectively. For the SC, SBM and mSBM diets, AID of CP and AA were associated with ileal recoveries of both endogenous N and dietary N. The latter were related to trypsin inhibitor activity in these diets. Properties of pea fibres negatively affected the AID of CP and AA and the recoveries of ileal endogenous N. The low AID of CP and AA in the pRH were caused by a high content of undigested non-protein N in the fibre fraction.

Key Words: Pigs; Ileal digestibility; Endogenous nitrogen; Soybeans; Pea; Rapeseed hulls

Introduction

A variety of feedstuffs, e.g., legume seeds, used in pig production, contain anti-nutritional factors (**ANF**), such as trypsin inhibitors (**TI**), lectin and tannins (Huisman and Jansman, 1991). These ANF may increase the amount of endogenous nitrogen (**N**) present at the terminal ileum of pigs (Huisman et al., 1992, Schulze, 1994; Jansman et al., 1995). Also the amounts and chemical-physical properties of dietary fibres, e.g., neutral detergent fibre and pectin, may increase ileal endogenous N losses (Lien et al., 1994; Mosenthin et al., 1994; Schulze et al., 1994, 1995; Leterme at al., 1996). These studies also showed that variation in apparent ileal digestibility of dietary protein is related to endogenous N at the terminal ileum. Depending on the level of ingestion, those aforementioned dietary factors can also affect the true ileal digestibility of dietary protein (Huisman et al., 1992; Schulze, 1994). For an efficient pig production, therefore, it is important to know more about the effects of feeds with high amounts of ANF and(or) fibres on the physiological responses of the animal.

Table 1. Chemical characteristic of the protein sources (g/kg DM)

Item	SC[a]	SBM[b]	mSBM[c]	Peas	pRH[d]
Dry matter	953.0	875.8	909.0	868.8	874.6
CP (N x 6.25)	709.3	530.8	555	227.2	166.5
Ether extract	8.4	22.8	136	12.7	81.6
Ash	72.4	72.3	74.7	39.8	56.5
Crude fibre	30.4	36.5	39.9	87.6	311.5
NDF[e]	ND[g]	73.7	75.7	161.4	528.1
ADL[f]	ND[g]	ND[g]	ND[g]	ND[g]	258.9
TIA (mg/g)[h]	2.5	3.7	14.5	2.4	ND[g]
Amino acids					
Arginine	47.6	40.2	42.7	1.86	6.8
Histidine	19.8	14.7	15.6	0.61	3.0
Isoleucine	33.2	26.0	26.9	0.97	6.6
Leucine	57.9	43.7	44.5	1.73	9.0
Lysine	46.5	33.0	37.9	1.62	10.1
Methionine	10.3	8.0	7.7	0.21	2.2
Cystine	11.1	8.2	8.8	0.33	4.0
Phenylalanine	37.1	29.8	33.3	1.12	6.1
Threonine	27.3	22.6	24.8	0.89	9.5
Tryptophan	9.2	6.4	7.2	0.19	1.6
Valine	35.1	27.8	30.9	1.19	9.8
Alanine	31.2	26.0	29.0	1.11	6.1
Aspartic acid	84.8	69.9	79.8	3.02	14.6
Glutamic acid	127.4	104.9	108.7	3.77	19.1
Glycine	31.5	24.5	26.2	1.06	6.8
Proline	45.3	35.0	69.4	1.31	13.8
Serine	35.7	28.7	31.9	1.10	8.0
Tyrosine	25.7	18.0	19.8	0.60	6.3

[a] Soybean concentrate.

[b] Toasted soybean meal.

[c] Mixture of toasted and untoasted soybean meal (ratio of 75:25, respectively).

[d] Purified rapeseed hulls.

[e] Neutral detergent fibre.

[f] Acid detergent fibre.

[g] Not determined.

[h] Activity of trypsin inhibitors (TIA) expressed as milligrams of trypsin inhibited per gram of dry matter of the product.

In the present study following feed products were used: (a) soybean products with different levels of trypsin inhibitors activity (**TIA**), (b) rapeseed hulls, as a dietary fibre source, and (c) peas, as a source of both ANF and fibres. The effects of these feed products on the apparent digestibility of protein and on the recoveries of endogenous and dietary N at the terminal ileum of piglets were investigated.

Materials and Methods

Feedstuffs

The test products used were: toasted and alcohol treated soybean concentrate (**SC**) as a reference protein source, with a low TIA, a commercial batch of soybean meal (**SBM**), a mixture of toasted and untoasted (75:25, respectively) soybean meal with a high TIA (**mSBM**), peas and purified rapeseed hulls (**pRH**). The pRH were prepared by CETIOM-GERDOC (Pessac, France) from of a low glucosinolate variety of rape seeds (*Brassica napus,* L.). The chemical composition of the test products is given in Table 1.

Animals and housing

The experiment was carried out using 13 crossbred barrows [(Dutch Landrace x Yorkshire) x Finnish Landrace]. Piglets were about seven to eight weeks old and had a body weight (**BW**) (± SE) of 11.5 kg (± 0.12 kg) at the arrival. They were individually housed in metabolism cages (80 x 180 cm) at an ambient temperature of 22 to 24°C and relative air humidity of about 55 percent. In the cages, animals could move freely. Before the first experimental period, piglets were randomly allocated to the dietary treatments (Table 2). The investigation was reviewed and approved by the TNO-Committee for Animal Welfare.

Diets and feeding

The SC, SBM, mSBM and peas were the sole protein sources in the maize starch-based diets (**SC diet**, **SBM diet** and **pea diet**, respectively; Table 2). In case of diet with pRH (**SRH diet**), the hulls were added as a fibre source [neutral detergent fibre (**NDF**)] to the SC diet at the expense of cellulose and SC. The diets contained 108 g/kg diet of calculated apparent ileal digestible (**ID**) CP (N x 6.25) and essential amino acids (**AA**). The amounts of apparent ID CP and ID AA in the diets were calculated based on the determined digestibility coefficients for similar diets in a

Table 2. The ingredient composition of the experimental diets (g/kg as fed-basis)

Item	Diet[a]				
	SC	SRH	SBM	mSBM	Pea
Soybean concentrate	182.0	137.9	-	-	-
Soybean meal	-		280.0	-	-
mSBM[b]	-		-	31.75	-
Purified rapeseed hulls	-	250.0	-	-	-
Peas	-	-	-	-	71.30
Corn starch	518.7	385.5	463.8	44.25	7.09
Dextrose	150.0	150.0	150.0	15.00	15.00
Soya oil	13.0	24.0	13.0	1.30	1.55
Cellulose	80.0	-	44.0	3.50	-
$CaCO_3$	11.6	10.5	12.0	1.20	1.35
$CaHPOH_2O$	18.0	16.0	17.5	1.70	1.50
NaCl	3.0	3.0	3.0	0.30	0.30
$KHCO_3$	7.5	7.5	1.0	-	0.10
$NaHCO_3$	2.5	2.5	2.5	0.25	0.25
MgO	0.5	-	-	-	0.05
L-lysine·HCl	-	0.5	0.3	-	-
DL-methionine	1.1	0.7	1.1	0.08	0.21
L-threonine	0.5	0.3	0.2	0.02	0.08
L-tryptophan	0.1	0.1	0.1	-	0.07
Cr_2O_3	1.5	1.5	1.5	0.15	0.15
Premix[c]	10.0	10.0	10.0	1.00	1.00

[a] The maize starch-based diets contained either soya concentrate (SC), rapeseed hulls added to the SC diet in expense of SC and cellulose (SRH), soybean meal (SBM), a mixture of toasted and untoasted soybean meal (mSBM), or peas as the protein sources.

[b] Mixture of 75% toasted soybean meal and of 25% untoasted soybean meal.

[c] The vitamin/mineral mixture supplied per kg feed: 9000 IU vit. A; 1800 IU vit. D_3; 40 mg vit. E; 5 mg riboflavin; 30 mg niacin; 12 mg pantothenic acid; 100 mg choline, 40 µg vit. B_{12}; 2 mg vit. B_1; 3 mg vit. B_6; 0.1 mg biotin; 1 mg folic acid; 3 mg vit. K; 50 mg ascorbic acid; 300 mg $ZnSO_4 \cdot H_2O$; 15 mg MnO_2; 400 mg $FeSO_4 \cdot 7H_2O$; 2.5 mg $CoSO_4 \cdot 5H_2O$; 0.5 mg KI; 35 mg $CuSO_4 \cdot 5H_2O$; 0.2 mg $Na_2SeO_3 \cdot 5H_2O$; 40 mg tylosin.

previous trial (Grala et al., unpublished data). The diets were supplemented with crystalline AA (lysine, threonine, methionine and tryptophan) to contain at least 75% of the requirements for the apparent ID AA for piglets of 10 to 25 kg BW (CVB, 1994). Chromic oxide (Cr_2O_3) was added to the diets as an indigestible marker (1.5

g/kg as-fed diet). The analyzed and estimated contents of nutrients in the diets are given in Table 3.

During the experimental periods, diets were fed four times a day in equal meals at 0200, 0800, 1400 and 2000 h. Pigs were individually fed at the level of 2.7 times the maintenance requirement for metabolizable energy (ME), (2.7 x 420 kJ ME per $kg^{0.75}$ per day; ARC, 1981). Water was available from drinking nipples from the start of feeding until 20 min after feed supply.

Table 3. Chemical composition of the experimental diets (%)

Item	Diet[a]				
	SC	SRH	SBM	mSBM	Pea
ME, MJ/kg[b]	14.4	14.4	14.4	14.4	14.4
Dry matter	883.1	907.6	919.5	92.89	91.54
CP (N x 6.25)	122.4	132.2	137.1	16.48	14.48
Ether extract	8.1	39.2	17.1	1.39	2.21
Crude fibre	58.0	72.3	40.0	3.65	5.41
Ash	48.3	54.9	49.3	5.14	5.75
NDF[c]	78.1	124.8	56.2	4.80	9.02
TIA (mg/g)[d]	4.0	-	9.5	4.28	1.57
ID-CP (N x 6.25)[e]	108.2	108.0	108.0	10.80	10.80
ID-Lysine	7.5	7.5	7.5	0.84	0.84
ID-Methionine	2.8	2.5	2.8	0.25	0.31
ID-Methionine+cystine	4.4	4.4	4.4	0.44	0.44
ID-Threonine	4.4	4.4	4.4	0.44	0.44
ID-Tryptophan	1.5	1.5	1.5	0.15	0.15
Lysine[f]	8.1	8.7	8.6	1.08	1.02
Methionine	2.9	2.7	3.1	0.30	0.35
Methionine+cystine	4.8	5.0	5.2	0.56	0.55
Threonine	5.5	6.0	5.4	0.69	0.60
Tryptophan	1.7	1.8	1.8	0.21	0.20

[a] For a diet description see footnote in Table 2.

[b] Net energy (NE) was calculated from its content in the ingredients.

[c] Neutral detergent fibre.

[d] Activity trypsin inhibitors (TIA) was calculated based on the analyzed TIA of the protein sources; expressed as milligrams of trypsin inhibited per gram product.

[e] Apparent ileal digestible (ID) crude protein (CP) and apparent ID amino acids (AA) were estimated based on the analyzed CP and AA contents of the protein sources that supplied the diets and then corrected for the apparent ileal digestibility values (Grala, personal communication).

[f] The amino acid contents in the diets were estimated from the analyzed contents in the protein sources.

	Preliminary period				Experimental period					
Day	0-10	9-11	10-20	20-23	1-7	8-10	11-17	18-20	21-27	28-30
	Adaptation	PVTC[a]	Recovery	Catheters[b]	Pre-period 1	Digesta collection 1	Pre-period 2	Digesta collection 2	Pre-period 3	Digesta collection 3
					[^{15}N]leucine infusion					

Figure 1. Scheme of the experimental procedure.

[a] Ileal cannulation using Post-Valve-T-Caecum (PVTC) cannula (Van Leeuwen et al., 1991)

[b] Insertion of two blood catheters into blood vessels; first one placed in the external jugular vein (for blood sampling), and the second - in the carotid artery (for the infusion of [^{15}N]leucine)

Experimental procedure

The experimental scheme is presented in Figure 1. After an adaptation period on a commercial diet (on average 10 days), pigs were surgically fitted with a Post-Valve-T-Caecum (PVTC) cannula according to Van Leeuwen et al. (1991). The recovery period (about 11 days) was followed by the insertion of two catheters into the blood vessels. The first catheter was placed in the external jugular vein (for blood sampling), and the second - in the carotid artery (for the infusion of [^{15}N]-Leucine).

Pigs were used in a changeover arrangement of treatments within three experimental periods. In each period, there were seven days for adaptation to dietary treatments and three successive days of digesta collections from 0800 to 2000 h. The first period started with a simultaneous introduction of experimental diets and with the beginning of the [^{15}N]-Leucine infusion. The continuous 30-d infusion of [^{15}N]-Leucine started one day after catheterization. The infusion rate of [^{15}N]-Leucine (99% atom ^{15}N; Euriso-Top, Belgium) was 4.2 mg per kilogram BW per day. The [^{15}N]-Leucine was dissolved in a sterile non-pyrogenic physiological saline solution (NaCl, 9 g/L) and infused by perfusion pumps (Fr. B. Braun Melsungen AG, Germany). The 'Swivel technique' was applied to infuse continuously the freely moving pigs (Van Kleef, 1996). Blood samples of about 8 mL (2 x 4 mL) were taken at the midpoint between feedings (1100 and 1700 h) on each day of digesta collection. After sampling, blood samples were treated as described by Schulze et al. (1995).

The apparent ileal digestibility (**AID**) of nutrients were determined using the chromic oxide ratio method (Furuya and Kaji, 1992). For the calculations of the AID of dietary CP (N x 6.25) and dietary AA, nitrogen form crystalline AA was assumed to be 100% absorbed before the end of the ileum. The ratio of ^{15}N-enrichment excess in ileal digesta to that in the trichloroacetic acid (TCA)-soluble blood plasma was determined. From this, endogenous N in ileal digesta was estimated using the following formula (De Lange et al., 1990):

$$N_e = N_d \times [(^{15}N_d - {}^{15}N_{diet(0)})/(^{15}N_{pl} - {}^{15}N_{pl(0)})] \qquad [1]$$

where: N_e is endogenous N in ileal digesta (g/kg DMI), N_d *is* total N in ileal digesta (g/kg DMI), $^{15}N_d$ is ^{15}N-enrichment of ileal digesta (%), $^{15}N_{diet(0)}$ is natural ^{15}N-enrichment of the diet (%), $^{15}N_{pl}$ is ^{15}N-enrichment in the TCA-soluble blood plasma (%), $^{15}N_{pl(0)}$ is natural ^{15}N-enrichment in the TCA-soluble blood plasma (%). True ileal digestibilities of CP for the diets were calculated by subtracting endogenous CP (N x 6.25) from total CP in ileal digesta.

The digestibility coefficients of CP and AA of pRH in the SRH diet were calculated by correcting the coefficients of the SRH diet for the presence of SC in this diet. These calculations referred as to so-called "difference method" using a following formula:

$$DC_{RH} = DC_{td} \times (100 \times N_{td} / G \times P) - [(100 \times N_{td} / G \times P) - 1] \times Dc_{bt} \qquad [2]$$

were: DC_{RH} is digestibility coefficient of CP or AA in pRH (%), DC_{td} is digestibility coefficient of CP or AA of the SRH diet (%), N_{td} is percentage of N or AA in the SRH diet, G is percentage of pRH in the SRH diet, P is percentage of N or AA in pRH, DC_{bt} is digestibility coefficient of CP or AA of the SC diet (%).

At the beginning of each 10-day experimental period, the average BW of pigs were 15.6 kg (± 0.19 kg), 18.5 kg (± 0.24) and 20.2 (± 0.24). At the end of the experiment the pigs' average BW was 23.1 kg (± 0.30 kg).

Chemical analyses

Samples of ileal digesta were pooled per pig within the experimental period. Nitrogen, DM and ether extract (EE) in the diets and in freeze-dried ileal digesta were analyzed according to standard methods (AOAC, 1984). Amino acids were analyzed as described by Andrews and Baldar (1985). Crude fibre (CF) was analyzed using standard method of NEN 5417 (1988). NDF was determined as described by Englyst and Cummings (1988). Chromium in the diets and ileal digesta was determined according to Bosch et al. (1988). The soya products and peas were analyzed for TIA according to

Table 4. Apparent ileal digestibility (%) of nutrients in the experimental diets

Item	Diet[a]					RMSE[b]
	SC	SRH	SBM	mSBM	Pea	
	n=6	n=7	n=7	n=7	n=5	
Dry matter	82.1^{d}	74.3^{f}	83.3^{d}	80.0^{e}	74.9^{f}	0.73
CP (N x 6.25)[c]	82.4^{d}	67.6^{f}	81.6^{d}	68.0^{f}	76.9^{e}	2.24
Ash	50.7^{d}	38.4^{e}	51.5^{d}	48.6^{d}	38.8^{e}	3.51
Organic matter	84.0^{d}	76.6^{f}	85.1^{d}	81.8^{e}	77.3^{f}	0.65
NDF	11.8^{e}	21.5^{d}	14.6^{e}	17.3^{de}	24.8^{d}	0.69

[a] For a diet description see footnote in Table 2.

[b] Root mean square of the error term (df = 31).

[c] Digestibility coefficients of crude protein (N x 6.25) are corrected for N added as crystalline AA, assuming their absorption of 100% before the end of the ileum.

[d,e,f] Means within a row lacking a common superscript letter differ at $P < 0.05$.

Van Oort et al. (1989). The ^{15}N-enrichment of ileal digesta, feed, and of the TCA-soluble fraction of blood plasma was determined according to the procedure described by Schulze et al. (1995).

Data analyses

The experimental arrangements were based on six to eight replications for each treatment. During period 1, due to disfunction of the jugular vein catheter, one animal (SC treatment) was replaced by another one of a similar genetic background and BW. Another pig did not eat the required amount of feed throughout the experimental periods 1, 2 and 3 (SBM, SC and pea treatments, respectively) and was discarded from data set. These data were treated as missing values. The numbers of observations obtained for each treatment were: six for the SC diet, seven for the SRH, SBM and mSBM diets, and five for the pea diet.

The effects of dietary treatments on the studied variables were analyzed using the GLM procedure of SAS (1990). Preceding analysis did not determine any Period x Animal interactions, therefore, main effects were analyzed using following model:

$$Y_{ijk} = \mu + T_i + P_j + A_k + e_{ijk} \qquad [3]$$

where: Y_{ijk} is dependent variable, μ is overall mean, T_i is treatment effect (i = 1, 2, 3, 4, 5), P_j is period effect (j = 1, 2, 3), A_k is animal effect, e_{ijk} is residual error. Differences among treatments were determined for the least-square means (LSM), using an adjustment for multiple comparisons by the Tukey-Kramer test (SAS, 1990).

Table 5. Apparent ileal digestibility (%) of amino acids in the experimental diets[a]

Item	Diet[b]					RMSE[c]
	SC	SRH	SBM	mSBM	Pea	
	n=6	n=7	n=7	n=7	n=5	
Arg	92.7^{d}	84.3^{ef}	91.4^{d}	81.9^{f}	89.0^{d}	1.80
His	88.0^{d}	76.5^{e}	86.6^{d}	74.6^{ef}	82.7^{d}	2.62
Ile	89.2^{d}	79.6^{e}	87.9^{d}	72.1^{f}	82.0^{e}	1.88
Leu	88.8^{d}	82.3^{e}	87.1^{d}	70.2^{f}	83.5^{e}	1.80
Lys	84.4^{d}	65.5^{f}	82.1^{d}	73.3^{e}	82.3^{d}	2.23
Met	89.3^{d}	74.5^{f}	90.0^{d}	77.5^{e}	73.6^{f}	1.70
Cys	78.5^{d}	57.5^{f}	80.2^{d}	60.2^{ef}	66.1^{e}	2.50
Phe	91.2^{d}	81.6^{e}	87.2^{de}	73.8^{f}	82.5^{e}	3.03
Thr	77.1^{d}	64.3^{f}	78.3^{d}	66.6^{ef}	70.8^{e}	2.88
Trp	86.0^{d}	73.2^{e}	86.0^{d}	73.2^{e}	67.1^{f}	2.88
Val	85.4^{d}	73.9^{ef}	84.3^{d}	69.8^{g}	78.0^{e}	2.12
Ala	82.7^{d}	76.9^{ef}	82.3^{de}	68.3^{f}	77.6^{de}	2.99
Asp	83.2^{de}	80.0^{e}	84.9^{d}	73.7^{f}	82.1^{de}	2.06
Glu	89.5^{d}	84.2^{e}	89.7^{d}	77.1^{f}	87.5^{de}	2.18
Gly	71.5^{d}	63.6^{de}	71.3^{d}	54.9^{e}	68.2^{d}	4.73
Pro	71.0^{d}	47.5^{d}	70.7^{d}	51.7^{d}	65.7^{d}	9.90
Ser	84.7^{d}	73.8^{e}	84.5^{d}	71.7^{e}	76.0^{e}	2.20
Tyr	89.9^{d}	75.0^{e}	86.0^{d}	72.7^{e}	77.8^{e}	3.29
Sum of AA	85.0^{d}	74.1^{e}	83.8^{d}	70.7^{e}	80.4^{d}	2.76
Non-AA N	63.0^{de}	22.8^{g}	65.6^{d}	36.4^{f}	50.8^{e}	6.32
AA N	85.2^{d}	74.0^{f}	84.0^{d}	71.3^{f}	80.9^{de}	2.75

[a] Digestibility coefficients of AA were corrected for N added as crystalline AA, assuming their absorption of 100% before the end of the ileum.

[b] For a diet description see footnote in Table 2.

[c] Root mean square of the error term (df = 31).

[d,e,f,g] Means within a row lacking a common superscript letter differ at $P < 0.05$.

Results

Apparent ileal digestibility of nutrients

There were differences among treatments ($P < 0.05$) in the AID of DM, CP, ash, OM and NDF (Table 4). The AID for DM, CP and ash in the SRH diet were about 7 to 15 percentage units lower than in the SC diet ($P < 0.05$) while the AID of NDF was higher by about 10 percentage units ($P < 0.05$). The pea diet had the lowest DM, ash and OM digestibilities, whereas it had the highest digestibility of NDF compared to the SC, SBM and mSBM diets, ($P < 0.05$). The AID of CP for the mSBM diet was 14.4, 13.6 and 8.9 percentage units lower ($P < 0.05$) than for the SC, SBM and pea diets, respectively.

There were differences among dietary treatments ($P < 0.05$) in the AID for all of the AA (Table 5) and corresponded with the differences in the AID of CP in those diets. The coefficients for the most essential AA (lysine, methionine, threonine and tryptophan) of the SRH diet were lower by 18.9, 14.8, 12.8 and 12.8 percentage units than those of the SC diet.

The digestibility coefficients of AA and N for the pRH (Table 6) were much lower than for the SRH diet. Of all of the AA in the pRH, the lowest digestibility value of 18% was calculated for lysine (48 percentage units lower than for the SRH diet). On average, the AID for the sum of AA was 33 percentage units lower in pRH than in the SRH diet. The AID of CP in pRH was calculated for 26 percent while the non-AA N was not digested to the end of the ileum.

^{15}N-enrichment of blood plasma and ileal digesta

The mean background ^{15}N-enrichment of the N in the TCA-soluble blood plasma and the diets were 0.3663 and 0.3671, respectively. There were no differences ($P > 0.05$) in the ^{15}N-enrichment excess of the N in blood plasma among treatments (Table 7). The ^{15}N-enrichment excess of the N in ileal digesta and the proportion of endogenous N to the total ileal N (dilution factor) differed among groups ($P < 0.05$). For the SRH diet, the ^{15}N-enrichment excess of the N in ileal digesta was lower ($P < 0.05$) by 61%, and dilution factor by 63% compared to the SC diet. The ^{15}N-enrichment excess of the N in ileal digesta and the dilution factors for pigsfed the mSBM and pea diets were lower ($P < 0.05$) than for those fed the SC diet.

Dry matter flow and recoveries of total, endogenous and dietary nitrogen at the terminal ileum

The flow of ileal DM, ileal recoveries of total N, endogenous N, dietary N and the true ileal digestibility of CP differed among treatments (Table 8). Both the DM flow and the recoveries of N in ileal digesta for the SC and SBM diets did not differ (P > 0.05). The flow of ileal DM for the SRH diet was almost 50% higher compared to the SC diet

Table 6. Apparent ileal digestibility (%) of crude protein (CP) and amino acids of purified rapeseed hulls in pigs[a]

Item	Digestibility coefficient[b]	SEM
Arginine	41	4.8
Histidine	28	3.1
Isoleucine	42	1.6
Leucine	48	2.8
Lysine	18	2.0
Methionine	46	2.5
Cystine	29	2.1
Phenylalanine	33	6.3
Threonine	34	2.0
Tryptophan	38	3.3
Valine	39	1.5
Alanine	48	4.3
Aspartic acid	58	1.7
Glutamic acid	53	1.6
Glycine	34	9.2
Proline	19	10.7
Serine	36	2.2
Tyrosine	39	3.6
CP (N x 6.25)	26	3.9
Non-AA N	(-1)	5.7
Sum of amino acids	41	2.2

[a] Digestibility coefficients for the purified rapeseed hulls were calculated by the difference between the SC diet and the diet containing rapeseed hulls added to the SC diet in expense of soya concentrate and cellulose (SRH diet).

[b] Digestibility coefficients for crude protein and amino acids (AA) were corrected for N added to the diets as crystalline AA, assuming their absorption of 100% before the end of the ileum.

Table 7. ^{15}N-enrichment excess[a] in the trichloroacetic acid (TCA)-soluble blood plasma and ileal digesta of pigs fed experimental diets

Item	Diet[b]					RMSE[c]
	SC	SRH	SBM	mSBM	Pea	
	n=6	n=7	n=7	n=7	n=5	
Blood plasma	0.0370	0.0358	0.0363	0.0326	0.0329	0.0029
Ileal digesta	0.0267^{e}	0.0164^{g}	0.0228^{f}	0.0181^{g}	0.0183^{g}	0.0020
Dilution factor[d]	0.727^{e}	0.463^{g}	0.627^{ef}	0.552^{fg}	0.557^{fg}	0.055

[a] The background ^{15}N-enrichment of the N in TCA-soluble blood plasma and feed were 0.3663 and 0.3672, respectively.

[b] For a diet description see footnote in Table 2.

[c] Root mean square of the error term (df = 31).

[d] Dilution factor equals ^{15}N-enrichment excess in ileal digesta / ^{15}N-enrichment excess of the N in the TCA-soluble blood plasma.

[e,f,g] Means within a row lacking a common superscript letter differ at $P < 0.05$.

Table 8. The flow of dry matter (DM) and recoveries of total nitrogen (N), endogenous N and dietary N in addition to the true digestibility of CP at the terminal ileum of pigs fed experimental diets

Item	Diet[a]					RMSE[b]
	SC	SRH	SBM	mSBM	Pea	
	n=6	n=7	n=7	n=7	n=5	
DM flow (g/kg DMI)	164.1^{ef}	244.2^{c}	154.3^{f}	193.2^{de}	219.5^{cd}	15.6
Nitrogen in ileal digesta						
Total (g/kg DMI)	3.95^{f}	7.57^{d}	4.37^{f}	9.04^{c}	5.82^{e}	0.55
Endogenous (g/kg DMI)	2.79^{d}	3.46^{d}	2.73^{d}	4.89^{c}	3.29^{d}	0.45
Dietary (g/kg DMI)	1.16^{e}	4.11^{c}	1.64^{e}	4.15^{c}	2.53^{d}	0.42
True ileal CP digestibility	94.9^{c}	82.4^{f}	93.1^{c}	85.5^{e}	89.9^{d}	1.70

[a] For a diet description see footnote in Table 2.

[b] Root mean square of the error term (df = 31).

[c,d,e,f] Means within a row lacking a common superscript letter differ when $P < 0.05$

($P < 0.05$). The DM flow for the pea diet was higher ($P < 0.05$) than for the mSBM diet, and the DM flows for these two treatments were higher ($P < 0.05$) than for the SC or the SBM diet.

The recovery of total ileal N for the SRH diet was higher ($P < 0.05$) than for the SC diet. It was mainly the effect of a higher ($P < 0.05$) recovery of dietary N. The recovery of total N in ileal digesta with the mSBM diet was the highest of all treatments ($P < 0.05$). Compared to the SC diet, the recovery of ileal endogenous N for the mSBM diet was 75% higher ($P < 0.05$), and the recovery of dietary N was 258% higher ($P < 0.05$). For the pea diet, the recovery of ileal endogenous N was 18% higher ($P > 0.05$) than for the SC diet and 33% lower ($P < 0.05$) than for mSBM diet. The recovery of ileal dietary N for the pea diet was 118% higher ($P < 0.05$) than for the SC diet, and 39% lower ($P < 0.05$) than for the mSBM diet.

The true ileal digestibilities of CP of the diets corresponded with the recoveries of dietary N in ileal digesta ($P < 0.05$). The highest coefficients were found for the SC and SBM diets ($P > 0.05$), followed by the pea, mSBM and SRH diets ($P < 0.05$).

Discussion

The purpose of the study on piglets was to determine the AID of CP and AA in semi-synthetic diets, containing soybean products with different TIA, peas and purified rapeseed hulls. Determination of the AID of CP and AA in the pRH also was the purpose of this study. Furthermore, it was aimed to measure ^{15}N-enrichment of N in ileal digesta and from that to estimate the effects of tested protein sources on the recoveries of endogenous N and dietary N.

The ^{15}N-dilution method was used to determine recoveries of ileal endogenous N at the terminal ileum of pigs. This method has been used to measure qualitative differences in recoveries (losses) of ileal endogenous N in pigs fed diets containing different protein sources (De Lange et al., 1990; Huisman et al., 1992; Schulze, 1994; Jansman et al., 1995; Grala et al., 1997).

Effects of purified rapeseed hulls on ileal digesta properties

Compared with the SC diet, an increased flow of ileal DM was observed with the SRH diet. Apparently, this was the effect of the inclusion of pRH, which contain a high amount of fibre (Table 1). Slominski and Campbell (1990) reported that rapeseed non-starch polysaccharides are poorly digested. Schulze et al. (1995) and Lenis et al. (1996) showed that the flow of ileal DM is dependent on the level of dietary NDF. Therefore, a higher NDF digestibility of the S[illegible] diet, compared to the SC diet, can be explained by the replacement of purified cellulose by pRH, in which NDF also consists of other carbohydrates, such as hemicellulose. Hemicellulose is considered to be highly fermented already by microflora in the pig's small intestine (Longland et al., 1988; Buraczewska et al., 1988; Laplace et al., 1989). Studies of Schulze et al. (1994) and Dierick et al. (1986) indicate that there may be a high microbial activity in the small intestine in terms of fiber fermentation.

Dietary fibers such as lignin, hemicellulose, cellulose and pectin, which are the main components of rapeseed hull carbohydrates (Bell, 1984), may also interact with the AA released during protein hydrolysis. This means that some AA may not be absored in the small intestine and pass into the large intestine (Bergner et al., 1981; Howard et al., 1986). This means that the recovery of ileal total N is increased, with a resulting decreased AID of CP and AA (Mosenthin et al., 1994; Lenis et al., 1996). It can be calculated from the AID of N in pRH (Table 6) that approximately three quarters of dietary N excreted in ileal digesta originated from the undigested CP of pRH. The recovery of ileal endogenous N increased to much smaller extent than dietary N. The amount of ileal endogenous N with the SRH diet could be moderated by the losses of intestinal mucin (Mariscal-Landin et al., 1995), sloughed off epithelial cells (Bjergegaard et al., 1991; Jin et al., 1994) and the adsorption of endogenous N to the pRH fibre.

The low ileal digestibilities of N and AA of the pRH (Table 6) can explain the reduced CP and AA digestibilities in the SRH diet. Moreover, it was calculated that the non-AA N in the pRH was not digested at all. This suggests that, in rapeseed hulls, a great part of N is mainly of non-protein origin, being strongly bound to the fibrous fraction (Mitaru et al., 1984; Bjergegaard et al., 1991). Therefore, digestive enzymes have a restricted access to both the cell wall components and the enclosed cell contents. In conclusion, it seems that the major effects of pRH on the N recovery at the terminal ileum was caused by the high fibre content in the hulls and by the poor digestibility of N/AA, bound to and(or) enclosed in the fibre.

Effects of soybean products and peas on ileal digesta properties

In soya beans, TI are the major ANF, which may reduce the nutritive value of soybean products for monogastric farm animals (Huisman and Jansman, 1991). In peas, combined effects both protease inhibitors (Huisman and Tolman, 1992) and chemical-physical properties of fibres may negatively influence the nutritive value for animals (Leterme et al., 1996).

According to Huisman and Tolman (1992), an activity of TI at the level of about 0.5 mg/g in the feed will probably not reduce nutrient's digestibility in pigs. The reduced AID of nutrients in ileal digesta of pigs fed the mSBM diet may be explained by a higher TIA of this diet (Table 2). In a previous experiment (Grala et al., unpublished data), in which the same batch of mSBM was used in a maize starch-based diet, similar effects of TIA were found on the AID of DM, CP and AA. Schulze (1994) also showed almost similar effects of incremental amounts of purified soybean TI in the diets on the digestibilities of CP in piglets. This may be caused by the ability of TI to decrease digestion and(or) reduce (re)absorption of both endogenous and dietary protein (Grant et al., 1993) and(or) by inactivating pancreatic enzymes (Liener and Kakade, 1980).

The present study, along with Schulze (1994) and Grala et al. (unpublished data), showed that with a high level of TIA (SC, SBM *vs* mSBM) the AID of CP was reduced more by increased ileal dietary N than by ileal endogenous N. This means that with the mSBM diet (TIA of 4.28 mg trypsin inhibited per gram diet), the substantial increase in the recovery of ileal dietary N was probably related to the TIA effect on the proteolytic enzymes. This conclusion agrees with findings of Schulze (1994) who showed in piglets that levels of TIA in the feed above 2.5 mg/g cause a negative effect on the true ileal digestibility of CP.

A markedly lower AID of nutrients in the pea diet, compared to the SC or SBM diets, can partly be explained by the replacement of a highly digestible corn starch by the hardly digestible pea fibres (non-starch polysaccharides). These results agreed with Lien at al. (1994), who showed a considerable reduction of AID for DM, CP and AA in diets with incremental levels of pea fibre. The reason for the highest NDF digestibility of the pea diet may similarly be explained as earlier for the SRH diet. However, different sources and various dietary levels of NDF might also influence the results.

The main effects on the ileal digesta with the pea diet seem to be depended on the presence of ANF (e.g., TI, lectin) and properties of the pea fibres (Lien et al., 1994; Leterme et al., 1996). Leterme et al. (1996) showed that the pea fibres (cotyledon inner fibre) have extremely high water-holding capacity. This property of the pea fibres may increase the passage of digesta in the small intestine (Jørgensen and Jensen, 1994). As a result, the flow of ileal DM and the recoveries of ileal N may be increased, and consequently, the DM, CP and AA digestibilities reduced (Decuypere et al., 1994;

Leterme et al., 1996).

The recovery of ileal endogenous N was not markedly higher with the pea diet, but the recovery of dietary N was approximately doubled compared with the SC and SBM diets. This indicates that a rather low TIA of the pea diet (1.57 mg/g diet) had a limited effect on the recovery of ileal endogenous N, and in contrast to the mSBM diet, there was a sufficient activity of proteolytic enzymes for protein digestion. Nevertheless, a markedly lower ileal CP digestibility of the pea diet, compared with the SC diet, may be due to associative effects of the aforementioned properties of the pea fibres.

Conclusions

The study showed that differences in ileal apparent digestibilities of protein and amino acids observed in piglets fed diets containing soybean products, peas and rapeseed hulls were related to different recoveries of both endogenous and dietary nitrogen. In the soybean products, trypsin inhibitors activity has a major impact on the digestion of a dietary protein and also affects losses of endogenous nitrogen at the terminal ileum. The presence of protease inhibitors and chemical-physical properties of fibres in peas may be both factors negatively effect the apparent ileal digestibilities of nutrients. Rapeseed hulls have negative effects on the apparent and true ileal digestibilities of dietary protein and amino acids. Ileal N digestibility of rapeseed hulls is low, because nitrogen is a structural component of the fibre fraction and is not digested.

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CHAPTER 5

EFFECTS OF ILEAL ENDOGENOUS NITROGEN LOSSES AND OF DIETARY AMINO ACID SUPPLEMENTATION ON NITROGEN RETENTION IN GROWING PIGS

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Effects of ileal endogenous nitrogen losses and of dietary amino acid supplementation on nitrogen retention in growing pigs

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Abstract

Ileal endogenous nitrogen (N) losses and N balance in growing pigs were investigated in two experiments with growing pigs of 40 to 60 kg. In Experiment 1, thirteen ileal cannulated barrows were fed diets based on maize starch, containing either soybean meal (SBM), with a low trypsin inhibitors activity (TIA), a mixture of toasted and untoasted soybean meal, with a high TIA (mSBM), a commercial batch of peas, or rapeseed expeller cake (RC). Ileal endogenous N recoveries were measured using the ^{15}N-isotope dilution technique. Apparent ileal digestibilities of crude protein (CP) for the SBM, mSBM, pea and RC diets were 82.8, 72.0, 76.7 and 68.7% ($P < 0.05$). True ileal CP digestibilities for these diets were 96.5, 93.0, 94.0 and 87.5% ($P < 0.05$), and the recoveries of ileal endogenous N were 3.08, 6.01, 4.55 and 5.36 g/kg DMI ($P < 0.05$). In Experiment 2, sixteen barrows were used to determine N balance. The diets contained either SBM, mSBM or peas and were balanced for the contents of apparent ileal digestible (ID) CP (96 g/kg) and ID essential amino acids (EAA; at least 85% of requirements). The fourth treatment was the mSBM diet, supplemented with EAA to the level of 95% of the requirements, but contained a similar amount of ID CP (mSBMs diet). Apparent faecal CP digestibilities for the SBM, mSBM, pea and mSBMs diets were 88.6, 87.2, 86.1 and 86.0% ($P < 0.05$). Urinary N excretion and N retention for these diets were 0.39, 0.59, 0.40, 0.53 ($P < 0.05$), and 0.87, 0.80, 0.85, 0.84 g/kg$^{0.75}$ per day ($P < 0.05$), respectively. Utilization of dietary ID N for N retention were 79.8, 73.3, 78.2 and 77.6% ($P < 0.05$), respectively. The study showed that higher ileal endogenous N losses are associated with higher losses of urinary N and with a lower N retention. Supplementation by AA of diets with high ileal endogenous N losses may compensate for N losses, resulting in a decreased urinary N excretion and an increased N retention in pigs.

Key Words: Pigs; Endogenous nitrogen losses; Ileal digestibility; Nitrogen balance

Introduction

Amino acids (**AA**), which are not absorbed before the end of the ileum, have little, or no value as sources for protein synthesis in the pig (Żebrowska et al., 1978). Endogenous N found at the terminal ileum represents only part of the total secretions (25%; Souffrant, 1991; Krawielitzki et al., 1996). An optimal pattern of dietary essential AA is required for an efficient protein deposition in the pig (Fuller et al., 1989; Wang and Fuller, 1989) along with a sufficient energy supply in the diet (Bikker, 1994).

Recycling of endogenous protein [secretion, reabsorption and (re)synthesis] depends on animal and dietary factors (Nyachoti et al., 1997). When endogenous nitrogen losses (**ENL**) will increase, these losses (first limiting AA) will be replenished at the expense of AA for growth (Fuller, 1991; Moughan, 1993). As a result, less AA will be used for N retention causing extra oxidation of AA that were in turn supplied in excess. These unbalanced AA will be catabolized, and resulting N will contribute to urinary N losses (Moughan, 1995; Sève and Henry, 1996). Therefore, enhanced ENL may induce greater urinary N losses. In this respect, Huisman et al. (1993) calculated that a part of urinary N may originate from the N losses during recycling of endogenous protein. Finally, the gretaer ENL may negatively affect efficiency of N utilization for retention in the pig.

The present study was undertaken to determine recoveries (losses) of endogenous N at the terminal ileum as a consequence of giving diets with four different protein sources to growing pigs (BW 40-60 kg). It was subsequently aimed to study the effects of ileal endogenous N losses on urinary N excretion and N retention in growing pigs when feeding diets balanced for apparent ileal digestible crude protein and AA. The effect of AA supplementation to compensate for endogenous N loss was determined, using the protein source resulted in the highest recovery of ileal endogenous N.

Materials and methods

Feedstuffs

The feedstuffs used in two experiments were: a commercial batch of soybean meal (**SBM**), a mixture of toasted and untoasted soybean meal (ratio of 75:25), with a high trypsin inhibitor activity (**mSBM**), a commercial batch of peas (*Pisum sativum*), and rapeseed expeller cake (**RC**). The RC was prepared by CETIOM-GERDOC (Pessac, France) from rape oilseeds (*Brassica napus,* L.) of a low glucosinolate variety. A detailed description of the technological processing of the mSBM and RC were reported by Grala et al. (1998a). The chemical characteristics of the feedstuffs are presented in Table 1.

Apparent ileal digestibility of nutrients and recovery of ileal endogenous N (Experiment 1)

Animals and housing

The experiment was performed using 13 crossbred barrows [(Dutch Landrace x Yorkshire) x Finnish Landrace], with a mean body weight (**BW**; ± SE) of 38.6 kg (± 0.5 kg) at the arrival day. Pigs were individually housed in metabolism cages (80 x 180 cm) at an ambient temperature of 22 to 24°C and air humidity of about 55 percent. The

Table 1. Analyzed chemical composition of soybean meal (SBM), a mixture of toasted and untoasted soybean meal (mSBM), peas and rapeseed cake (RC) (g/kg DM)

Item	SBM	mSBM[a]	Peas	RC
Dry matter	881.0	895.0	879.0	947.0
CP (N x 6.25)	533.5	550.8	236.6	356.9
Ether extract	20.5	13.6	23.0	122.5
Ash	72.2	73.6	37.7	71.8
Crude fibre	39.6	39.9	67.9	153.1
NDF[b]	74.1	80.0	122.5	241.8
TIA (mg/g of product)[c]	3.62	14.55	1.80	ND[d]
Amino acids:				
Arginine	40.3	42.2	19.7	21.1
Histidine	14.8	14.6	5.3	9.2
Isoleucine	25.0	25.8	9.6	14.9
Leucine	41.5	43.4	16.6	25.8
Lysine	33.0	36.3	15.9	18.4
Methionine	7.6	7.5	2.4	6.4
Cystine	8.4	8.7	4.2	8.1
Phenylalanine	27.2	29.7	11.5	12.1
Threonine	21.8	22.9	8.9	17.3
Tryptophan	6.9	7.5	2.2	4.6
Valine	26.4	28.7	11.5	21.1
Alanine	24.0	24.2	10.5	16.8
Aspartic acid	64.2	64.6	25.9	27.7
Glutamic acid	105.3	114.6	46.1	58.1
Glycine	23.5	24.9	11.0	19.1
Proline	28.3	34.3	10.9	21.8
Serine	28.4	29.7	11.7	18.0
Tyrosine	18.8	20.1	8.0	11.8

[a] Composed of a mixture of 75% toasted soybean meal and of 25% untoasted soybean meal.

[b] Neutral detergent fibre.

[c] Trypsin inhibitor activity (TIA) expressed as milligrams of trypsin inhibited per gram of dry matter of the product.

[d] Not determined.

animals could move freely in the cages. The experiment was reviewed and approved by the TNO Committee for Animal Welfare.

Diets and feeding

The feed products - SBM, mSBM, peas and RC - were the sole protein sources in the maize starch-based diets and provided 104 g of calculated apparent ileal digestible (ID) CP (N x 6.25) per kilogram as-fed diet (Table 2). The diets were supplemented with crystalline AA (lysine, threonine, methinine, tryptophan) to contain at least 85% of the requirements for apparent ID essential AA (lysine, methionine+cystine, threonine and tryptophan) for fattening pigs (CVB, 1995). Lysine was the first limiting ID AA. The level of ID both CP and AA in the diets were calculated based on the digestibility coefficients in a previous experiment (Grala et al., 1997a). The diets were pelleted under a low temperature condition (<60 °C). Chromic oxide (Cr_2O_3) was added to the diets as an indigestible marker (1.5 g/kg diet).

Pigs were individually fed four times a day in equal meals at 0800, 1400, 2000 and 0200 h, using automated feed dispensers. The feeding level was 2.7 times the metabolizable energy (ME) requirement for maintenance [2.7 x 419 kJ per unit of a metabolic BW ($kg^{0.75}$); ARC, 1981]. Water was available from drinking nipples from the start of feeding until 35 min after the feed supply.

Experimental procedure

After an adaptation period of seven days, thirteen pigs were surgically fitted with a Post-Valve-T-Caecum cannula (Van Leeuwen et al., 1991). Then, following a recovery period of about nine days, two catheters were inserted; one in the external jugular vein and one in the carotid artery. During the recovery period, the barrows were offered incremental amounts of a commercial feed until they reached the desired feeding level. Three days after the catheters' insertion, the [^{15}N]leucine infusion started and the pigs were assigned to four experimental treatments in a balanced two-period changeover design. In each of these two periods, there were nine days for adaptation to the dietary treatments and three successive days of digesta collections from 0800 to 2000 h. Ileal digesta were hourly collected in plastic bags fixed to the cannula and frozen at -20 °C.

The infusion of [^{15}N]leucine (99% atom ^{15}N; Euriso-Top, Belgium) lasted continuously for 24 days in a rate of 4.2 mg per kilogram BW per day. The [^{15}N]leucine was dissolved in a sterile non-pyrogenic physiological saline solution (NaCl, 9 g/L) and infused via perfusion pumps (Fr. B. Braun Melsungen AG, Germany). The 'Swivel

Table 2. The ingredient composition of the experimental diets (g/kg as-fed basis)

Item	Experiment 1				Experiment 2			
	SBM	mSBM	Pea	RC	SBM	mSBM	Pea	mSBMs[a]
Soybean meal	293.0	-	-	-	251.5	-		
mSBM[b]	-	315.0	-	-		256.7		256.7
Peas	-	-	692.5	-		-	555.9	
Rapeseed cake	-	-	-	468.0				
Maize starch	439.4	427.1	88.5	310.5	471.0	466.1	195.8	466.9
Dextrose	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Soya oil	13.0	13.0	20.0	27.5	13.0	13.0	13.0	13.0
Cellulose	57.0	47.0	-	-	66.5	52.5	22.5	52.5
Vitamin/mineral	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
NaCl	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
$CaCO_3$	12.0	12.0	13.5	7.5	12.0	12.0	13.5	12.0
$CaHPO{\cdot}H_2O$	17.0	17.0	14.5	13.5	17.0	17.0	14.5	17.0
MgO	-	-	0.5	-	-	-	0.5	-
$NaHCO_3$	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$KHCO_3$	1.0	1.0	1.0	4.5	2.5	2.5	2.5	2.5
Cr_2O_3	1.5	1.5	1.5	1.5	-	-	-	-
L-lysine·HCl	-	-	-	1.5	0.05	-	-	0.95
DL-methionine	0.6	0.9	1.7	-	0.95	1.16	1.59	1.40
L-threonine	-	-	0.2	-	-	0.52	0.82	0.98
L-tryptophan	-	-	0.6	-	-	0.21	0.66	0.35
L-isoleucine	-	-	-	-	-	-	0.10	0.05
L-leucine	-	-	-	-	-	-	0.20	0.52
L-valine	-	-	-	-	-	0.15	0.36	0.70
L-cystine	-	-	-	-	-	0.20	0.06	0.41
L-glutamic acid	-	-	-	-	-	11.00	11.00	7.00
L-glycine	-	-	-	-	--	1.50	1.50	1.50

[a] The mSBMs diet is the mSBM diet, which is extra supplemented with the essential amino acids (AA) to compensate for the losses of AA during recycling of endogenous protein.

[b] Composed of a mixture of 75% toasted soybean meal and of 25% untoasted soybean meal.

[c] The vitamin/mineral mixture supplied per kg feed: 9000 IU vit. A; 1800 IU vit. D_3; 40 mg vit. E; 5 mg riboflavin; 30 mg niacin; 12 mg pantothenic acid; 100 mg choline, 40 µg vit. B_{12}; 2 mg vit. B_1; 3 mg vit. B_6; 0.1 mg biotin; 1 mg folic acid; 3 mg vit. K; 50 mg ascorbic acid; 300 mg $ZnSO_4{\cdot}H_2O$; 15 mg MnO_2; 400 mg $FeSO_4{\cdot}7H_2O$; 2.5 mg $CoSO_4{\cdot}5H_2O$; 0.5 mg KI; 35 mg $CuSO_4{\cdot}5H_2O$; 0.2 mg $Na_2SeO_3{\cdot}5H_2O$; 40 mg tylosin.

technique' was applied to infuse continuously the freely moving pigs (Van Kleef, 1996). Blood samples of about 8 mL (2 x 4 mL) were taken on each day of digesta collection at the midpoints between feedings (1100 and 1700 h). Thereafter, blood was treated as described by Schulze et al. (1995).

The apparent ileal digestibility (**AID**) of DM, ash, CP and AA were determined using the chromic oxide ratio method (Furuya and Kaji, 1992). For the AID calculations of CP and AA, corrections have been made for N added to the diets in a form of crystalline AA. These AA were assumed to be 100% absorbed before the end of the ileum. The ratio of ^{15}N-enrichment excess in ileal digesta to that in the trichloroacetic acid (**TCA**)-soluble blood plasma was determined. Then, the proportion of endogenous N in ileal digesta was estimated according to De Lange et al. (1990). At the start of the experimental period (beginning of the [^{15}N]leucine infusion), the average BW of pigs was 43.5 kg (± 0.4 kg) and at the end of the experiment 53.7 kg (± 0.5 kg).

Nitrogen balance (Experiment 2)

Animals and housing

The experiment was performed using 16 crossbred barrows [(Dutch Landrace x Yorkshire) x Finnish Landrace] that had a mean BW of 42.8 kg (± 0.3 kg) at the arrival day. Pigs were individually housed in metabolism cages at an ambient temperature of 22 to 24°C and a relative air humidity of about 50 percent. The cages were made of galvanized steel and allowed the collection of urine via underneath situated funnels.

Diets and feeding

The SBM, mSBM and peas were the only protein sources in the maize starch-based diets (Table 3) and originated from the same batches as those used in Experiment 1. The diets were formulated to contain similar amounts of apparent ID CP (96 g/kg as-fed diet). The diets were: **SBM diet**, with a low recovery of ileal endogenous N, **mSBM diet**, with a high recovery of ileal endogenous N and **pea diet** with an intermediate recovery of ileal endogenous N compared with the other diets. Diets were supplemented with crystalline AA to contain at least 85% of the requirements for apparent ID, essential AA for growing pigs (35 to 70 kg BW). Lysine was the first limiting ID AA. The fourth diet was an **mSBMs diet**, which was similar to the mSBM diet, but it had been supplemented with crystalline, essential AA up to 95% of the requirements for

Table 3. Chemical composition of the experimental diets (g /kg as-fed basis)

Item	Experiment 1				Experiment 2			
	SBM	mSBM	Pea	RC	SBM	mSBM	Pea	mSBMs
Dry matter	904.8	910.9	891.0	908.8	877.4	890.2	872.8	885.4
CP (N x 6.25)	146.5	162.5	146.6	161.1	124.7	139.1	126.1	140.0
Ether extract	25.3	23.4	37.4	86.7	17.9	16.4	24.5	16.4
Ash	48.3	50.9	54.2	55.4	45.6	46.6	47.4	46.3
Crude fibre	43.6	37.8	40.2	47.3	49.2	40.6	40.0	39.9
NDF[a]	68.5	55.5	60.7	90.7	72.1	60.9	73.1	62.7
TIA (mg/g of product)[b]	0.96	4.18	1.11	-	0.80	3.32	0.87	3.31
ME (MJ/kg)[c]	14.4	14.4	14.4	14.4	14.4	14.4	14.4	14.4
Indispensable amino acids[d]								
Lysine	8.0	10.2	9.6	8.9	7.4	8.4	7.8	9.1
Methionine	2.5	3.0	2.9	2.7	2.6	2.9	2.8	3.1
Methionine+cystine	4.5	5.4	4.9	6.1	4.5	5.1	4.9	5.6
Threonine	5.5	6.7	5.5	7.3	4.9	5.8	5.2	6.3
Tryptophan	1.6	2.0	1.7	2.0	1.5	1.9	1.7	2.1
Arginine	9.7	11.5	11.1	8.9	9.0	9.7	9.7	9.7
Histidine	3.6	4.2	3.6	3.9	3.3	3.4	2.6	3.4
Isoleucine	6.3	7.3	5.8	6.3	5.6	6.0	4.8	6.0
Leucine	10.6	12.1	10.3	10.9	9.3	10.1	8.4	10.6
Phenylalanine+tyrosine	11.6	14.4	10.2	10.0	10.3	11.5	9.6	11.5
Valine	6.7	8.4	7.1	8.8	5.9	6.8	6.0	7.3
ID-CP (N x 6.25)[e]	103.9	103.8	103.8	103.8	95.8	95.8	96.1	95.8
ID-Lysine	6.5	7.5	7.8	6.6	6.4	6.4	6.4	7.1
ID-Methionine	2.3	2.5	2.6	2.3	2.4	2.4	2.4	2.6
ID-Methionine+cystine	3.9	3.9	3.9	4.7	3.7	3.7	3.7	4.2
ID-Threonine	4.3	4.5	3.9	4.6	3.8	3.8	3.8	4.3
ID-Tryptophan	1.4	1.4	1.3	1.4	1.3	1.3	1.3	1.4
ID-Arginine	8.8	9.5	9.9	7.5	8.3	8.1	8.6	8.1
ID-Histidine	3.1	3.2	3.0	3.1	2.9	2.6	2.1	2.6
ID-Isoleucine	5.5	5.3	4.7	4.5	4.8	4.2	3.8	4.3
ID-Leucine	9.3	8.5	8.6	8.8	8.1	7.1	6.8	7.6
ID-	10.0	10.6	8.3	7.5	8.8	8.2	7.6	8.2
ID-Valine	5.6	5.9	5.4	6.3	5.0	4.6	4.6	5.2

[a] Neutral detergent fibre. [b] Trypsin inhibitors activity (TIA) was calculated from its content in the protein source. [c] Metabolizable energy (ME) was calculated from its content in the ingredients. [d] The contents of amino acids (AA) were calculated from their analyzed contents in the feed ingredients. [e] In Experiment 1, the diets contained at least 85% of the requirements for the most essential ileal digestible (ID) AA (lysine, methionine+cystine, threonine and tryptophan) in feeds for growing pigs (CVB, 1995). In Experiment 2, the SBM, mSBM and pea diets were supplemented with crystalline AA to contain at least 85% of required amounts of apparent ID, essential AA in feeds for growing pigs. The mSBMs diet has been extra supplemented with crystalline AA up to 95% of the requirements for ID essential AA to compensate for the AA losses, during recycling of endogenous proteins.

limiting, essential AA. With this extra AA supplementation, it was aimed to compensate for AA losses associated with an increased recycling of endogenous protein, when using mSBM as a protein source. The levels of apparent ID CP and essential AA in the diets were calculated based on the digestibility coefficients, determined in Experiment 1 (Table 4). The requirements for apparent ID lysine, methionine and cystine, threonine and tryptophan were according to CVB (1995) and for the other essential AA, according to the combined data of ARC (1981), Fuller et al. (1989), Wang and Fuller (1989), Boisen (1993), Lenis et al. (1993), Easter et al. (1995), Lewis and Nishimura (1995).

The diets were pelleted under a low temperature condition (<65°C). The feeding level was 2.7 times the ME requirement for maintenance [2.7 x 419 kJ ME per unit of a metabolic BW ($kg^{0.75}$); ARC, 1981]. Water was available from drinking nipples from the start of feeding until 20 min after the feed supply.

Experimental procedure

After an adaptation period (10 days) on a commercial diet, the pigs were assigned to four experimental treatments in a balanced two-period changeover design. Each of these two periods had a 12-day adaptation period and a 5-day N balance period. In the N balance periods, total faeces were collected in plastic bags attached to the pigs (Van Kleef et al., 1994). Bags were changed twice a day, frozen and kept at -20°C until faeces analyses. Urine was collected in plastic containers via funnels underneath the cages. Urine was removed once a day and stored at 4°C until analyses. To prevent N losses during collection and storage, 8 mL of 25% H_2SO_4 was added to each container every day. The average BW of barrows at the beginning of each N balance periods were 45.8 kg (± 0.5 kg) and 55.6 kg (± 0.6 kg).

The faecal apparent digestibilities of DM, organic matter (OM), CP and ash for the diets were calculated. For the calculations of the faecal apparent digestibility of CP, similar correction were made for N of crystalline AA N in the diets as in Experiment 1. Nitrogen intake and N excretion in faeces and urine were measured during the N balance periods. Then, N retention was calculated for each pig. The results had been expressed in grams per unit of a metabolic BW ($kg^{0.75}$) per day.

Chemical analyses.

In Experiment 1, samples of ileal digesta from 3-day collections in each experimental period were pooled per pig. In Experiment 2, samples of both faeces and

Table 4. Apparent ileal digestibilities (%) of dry matter, crude protein (CP), organic matter, ash and amino acids in fattening pigs fed maize starch-based diets containing either soybean meal (SBM), a mixture of toasted and untoasted soybean meal (mSBM), peas or rapeseed cake (RC) as the sole protein source (Experiment 1)

Item	Diet				RMSE^{a}
	SBM	mSBM	Pea	RC	
Dry matter	78.8^{c}	77.7^{cd}	76.3^{d}	71.5^{e}	1.0
CP (N 6.25)^{b}	82.8^{c}	72.0^{e}	76.7^{d}	68.7^{e}	2.2
Organic matter	81.1^{c}	79.8^{cd}	78.7^{d}	74.5^{e}	0.8
Ash	38.1^{c}	42.2^{c}	38.3^{c}	24.8^{d}	4.0
Arginine	92.5^{c}	85.0^{e}	89.3^{d}	83.0^{f}	0.8
Histidine	87.7^{c}	78.9^{d}	81.6^{de}	77.6^{e}	2.0
Isoleucine	86.5^{c}	74.7^{e}	79.9^{d}	76.3^{de}	1.8
Leucine	86.5^{c}	74.2^{e}	80.7^{d}	80.2^{d}	1.9
Lysine	85.2^{c}	78.2^{d}	81.5^{cd}	69.8^{e}	2.2
Methionine	86.3^{c}	76.2^{d}	70.2^{e}	86.5^{c}	3.1
Cystine	76.0^{c}	60.8^{e}	63.4^{de}	65.3^{d}	3.6
Phenylalanine	85.4^{c}	74.5^{e}	79.8^{d}	75.6^{de}	2.2
Threonine	77.8^{c}	67.1^{d}	70.9^{d}	68.2^{d}	3.1
Tryptophan	82.1^{c}	66.2^{de}	58.2^{e}	70.1^{d}	4.3
Valine	84.1^{c}	71.6^{e}	76.7^{d}	74.5^{de}	2.1
Alanine	81.5^{c}	71.0^{e}	75.5^{de}	75.7^{d}	2.4
Aspartic acid	84.9^{c}	74.4^{de}	80.5^{cd}	70.2^{e}	4.2
Glutamic acid	88.4^{c}	80.9^{de}	85.3^{cde}	82.2^{d}	2.4
Glycine	73.5^{c}	62.8^{d}	68.6^{cd}	65.3^{d}	4.4
Proline	75.9^{c}	66.2^{cd}	68.6^{cd}	56.1^{d}	5.4
Serine	85.0^{c}	72.1^{d}	75.6^{d}	71.4^{d}	2.9
Tyrosine	84.1^{c}	74.0^{de}	79.5^{cd}	70.4^{e}	3.2
Amino acid sum	85.1^{c}	74.8^{d}	79.1^{d}	74.2^{d}	2.7
Amino acid N	85.5^{c}	75.6^{de}	79.9^{d}	74.4^{e}	2.5
Non-amino acid N	56.2^{c}	59.9^{c}	57.0^{c}	41.4^{d}	3.2

[a] Root mean square of the error term (df = 23).

[b] Ileal digestibilities of crystalline amino acids, added to the diets, were assumed to be 100 percent.

[c,d,e,f] Means within a row lacking a common superscript letter differ at $P < 0.05$.

urine were pooled per pig for the entire 5-day N balance period within each experimental period. In both experiments, N and DM in the diets, freeze-dried ileal digesta and faeces, and N in urine were analyzed according to standard methods (AOAC, 1984). Amino acids were analyzed accoding to methods decribed by Adrews and Baldar (1985). Neutral detergent fibre (NDF) was determined as described by Englyst and Cummings (1988). Chromium in the diets and ileal digesta was determined according to Bosch et al. (1988). The activity of trypsin inhibitors (**TIA**) in soybean products and peas was analyzed according to a modified method of Kakade (Van Oort et al., 1989). The ^{15}N-enrichments of the N in ileal digesta, feed, and in the trichloroacetic acid (TCA)-soluble fraction of blood plasma were determined according to the procedures described by Schulze et al. (1995).

Statistical analysis

In both experiments, a balanced two-period changeover design with four treatments was used according to Gill and Magee (1976). In period 2 of Experiment 1, two pigs (one of the mSBM treatment and one of the RC treatment) and in period 1 of Experiment 2, one pig of the pea treatment did not maintain the required feeding level. The results appeared erroneous, therefore, data of those pigs were discarded and treated as missing values. Statistical analysis of data in both experiments was performed using the GLM procedure of SAS (1990). The effects of dietary treatment, animal and period on the determined variables in ileal digesta were analyzed according to the following linear model:

$$Y_{ijk} = \mu + T_i + A_j + P_k + e_{ijk}, \qquad [1]$$

where Y_{ijk} is a dependent variable, μ is an overall mean, T_i is the treatment effect (i = 1, 2, 3, 4), A_j is the animal effect (j = 1, 2,... 13 for Experiment 1, and j = 1, 2,...16 for Experiment 2), P_k is the period effect (k = 1, 2), and e_{ijk} is the residual error. The differences among treatments were determined for the least-square means (LSM), using an adjustment for multiple comparisons by the Tukey-Kramer test (SAS, 1990).

Results

Experiment 1

Apparent ileal digestibilities for DM, OM, ash, CP and AA differed among diets ($P < 0.05$; Table 4). The SBM diet had the highest CP digestibility followed by the pea

Table 5. Ileal flow of dry matter (DM) and endogenous nitrogen (N) and the true ileal digestibility of protein of fattening pigs fed maize starch-based diets, containing either soybean meal (SBM), a mixture of toasted and untoasted soybean meal (mSBM), peas or rapeseed cake (RC) as the sole protein source (Experiment 1)

	Diet				RMSE[a]
	SBM	mSBM	Pea	RC	
Ileal DM flow (g/kg DM intake)	268.2^{b}	286.2^{bc}	295.4^{c}	365.3^{d}	13.7
Endogenous N					
gram per kg DM intake	3.08^{b}	6.01^{d}	4.55^{bcd}	5.36^{cd}	0.61
gram per 100 g CP intake	13.8^{b}	21.1^{c}	17.3^{bc}	18.8^{c}	2.13
gram per gram of total ileal N	0.80^{b}	0.75^{b}	0.74^{b}	0.61^{c}	0.04
True ileal CP digestibility (%)	96.5^{b}	93.0^{c}	94.0^{bc}	87.5^{d}	1.40

[a] Root mean square of the error term (df=23).

[b,c,d] Means within a row lacking a common superscript letter differ at $P < 0.05$.

diet and the mSBM and RC diets. Differences in digestibility coefficients forindividual AA corresponded with the coefficients for CP in these diets. The AID for DM and OM for the SBM diet were higher than for the pea or RC diets ($P < 0.05$). The RC diet had the lowest ash digestibility compared with other diets ($P < 0.05$).

The amount of endogenous N and the true CP digestibility in ileal digesta differed markedly ($P < 0.05$) among treatments (Table 5). The SBM diet gave the lowest recovery of ileal endogenous N and the highest true ileal digestibility of CP compared with the other diets ($P < 0.05$). The highest recovery of ileal endogenous N was measured for the mSBM diet and was associated with a lower true ileal digestibility of CP compared with the SBM diet ($P < 0.05$). The true ileal digestibility of CP for the RC diet was 87.5% and was 5.5 to 9.0 percentage units lower than for the other diets ($P < 0.05$).

Experiment 2

Apparent faecal digestibilities of DM, OM and ash for the pea diet were lower ($P > 0.05$) compared with the SBM, mSBM and mSBMs diets (Table 6). The faecal digestibility of CP for the SBM diet was higher than for the pea and mSBMs diets ($P < 0.05$) and tended to higher ($P = 0.06$) for the mSBM diet. The faecal CP digestibility of the mSBM, pea and mSBMs diets did not differ ($P > 0.1$).

Nitrogen intake, faecal N, urinary N and N retention were calculated per $kg^{0.75}$ per day to correct for the differences in body weights of pigs in period 1 and 2 (Table 7). The

Table 6. Apparent faecal digestibilities (%) of dry matter, crude protein (CP), organic matter and ash in the experimental diets (Experiment 2)

Item	Diet[a]				RMSE[b]
	SBM	mSBM	Pea	mSBMs[c]	
Dry matter	93.7[e]	93.7[e]	92.0[f]	93.3[e]	1.1
CP (N x 6.25)[d]	89.9[e]	87.1[ef]	84.8[f]	86.2[f]	2.1
Organic matter	95.4[e]	95.5[e]	94.0[f]	95.1[ef]	1.0
Ash	62.8[e]	62.7[e]	57.0[e]	61.4[e]	4.5

[a] The diets were based on maize starch and contained either soybean meal (SBM), a mixture of toasted and untoasted soybean meal (mSBM) or peas as the sole protein source.

[b] Root mean square of the error term (df = 30).

[c] The mSBMs diet is the mSBM diet, which was extra supplemented with the essential amino acids (AA) to compensate for the losses of AA during recycling of endogenous protein.

[d] Ileal digestibilities of crystalline AA added to the diets were assumed to be 100 percent.

[e,f] Means within a row lacking a common superscript letter differ at $P < 0.05$.

N intake for the experimental groups was different ($P < 0.05$) and ranged from 1.41 g/kg$^{0.75}$/day for the SBM to 1.58 g/kg$^{0.75}$/day for the mSBMs diet. The estimated means for ID N intake did not differ ($P > 0.05$) among treatments [1.08 g/kg$^{0.75}$/d (± 0.003); data not shown]. The excretion of faecal N for the SBM diet was lower than for both diets with mSBM, or peas as a protein sources ($P < 0.05$). The lowest total excretion of urinary N were found for the SBM and pea diets and was highest for the mSBM diet ($P < 0.05$). For the mSBMs diet total urinary N tended to be lower than for the mSBM diet ($P = 0.08$) and was higher than for the other two diets ($P < 0.05$). After correction for the N absorbed from the large intestine, the excretion of urinary N for the mSBM diet remained 17, 13 and 9% higher than for the SBM, pea and mSBMs diets, respectively. The highest N retention was found for pigs fed the SBM diet and differed from those fed the mSBM or pea diet ($P < 0.05$). Nitrogen retention for pigs fed the mSBMs diet, with extra supplemented essential AA, did not differ ($P > 0.05$) from those fed the SBM, mSBM or pea diets.

The utilization of ingested N for retention in pigs fed the SBM was markedly higher than in those fed the pea, mSBM or mSBMs diet ($P < 0.05$). The utilization of ID N for retention differed between the SBM and pea diets ($P < 0.05$), and tended to differ between the SBM and mSBM diets ($P = 0.09$). For the mSBMs diet, the utilization of ID N for retention was 2.7 percentage units higher than for the mSBM diet ($P > 0.05$) and only 1.9 percentage units lower than for the SBM ($P > 0.1$).

Table 7. Nitrogen balance in pigs fed maize starch-based diets that were balanced for apparent ileal digestible protein and essential amino acids and caused different excretion of ileal endogenous N (Experiment 2)

Item	Diet[a]				RMSE[b]
	SBM	mSBM	Pea	mSBMs[c]	
N intake (g/kg$^{0.75}$/d)	1.41^{f}	1.58^{g}	1.43^{f}	1.58^{g}	0.012
N faeces (g/kg$^{0.75}$/d)	0.14^{f}	0.19^{g}	0.19^{g}	0.20^{g}	0.031
N urine (g/kg$^{0.75}$/d)	0.40^{f}	0.57^{g}	0.42^{f}	0.53^{g}	0.043
- absorbed from LI (g/kg$^{0.75}$/d)[d]	0.10	0.22	0.11	0.21	Nd[i]
- corrected (g/kg$^{0.75}$/d)[e]	0.30	0.35	0.31	0.32	Nd[i]
N retention (g/kg$^{0.75}$/d)	0.87^{f}	0.82^{g}	0.82^{g}	0.85^{fg}	0.038
N retention/intake (%)	61.5^{f}	52.1^{h}	56.9^{g}	53.6^{gh}	2.71
N retention/ileal digestible (%)	80.1^{f}	75.5^{fg}	74.7^{g}	78.2^{fg}	3.76

[a] The diets were based on maize starch and contained either soybean meal (SBM), a mixture of toasted and untoasted soybean meal (mSBM) or peas as the sole protein source.

[c] The mSBMs diet is the mSBM diet, which was extra supplemented with the essential amino acids (AA) to compensate for the losses of AA during recycling of endogenous protein.

[b] Root mean square of the error term (df = 30).

[d] Estimated N excretion in urine related to the fermentation of protein in the large intestine (LI); calculated from the difference of apparent faecal and ileal N digestibilities of the diets (Tables 6 and 4, respectively). The values are corrected for the presence of crystalline AA in the diets (assumed to be 100% absorbable before the end of the ileum).

[e] Corrected N excretion in urine; calculated from the difference between the total N excretion in urine and the estimated N excretion related to the fermentation of undigested dietary protein in LI (see footnote [4]).

[f,g,h] Means in a row lacking a common superscript letter differ at $P < 0.05$.

[i] Not determined.

Discussion

Effects of protein sources on nutrient digestibilities and recovery of endogenous nitrogen in ileal digesta

The ^{15}N-dilution technique was used to measure the recovery of endogenous N and to calculate the true digestibility of dietary protein at the terminal ileum of pigs (e.g., Huisman et al., 1992; De Lange et al., 1990; Schulze, 1994; Jansman et al., 1995; Grala et al., 1997b).

Apparent ileal digestibilities of CP and most of the AA for the diets with commercial batches of SBM and peas (Table 4) were within the range of values given by CVB (1995) and of a previous study (Grala et al., 1997a). In the latter study, the same batches of SBM, peas and mSBM were used in diets for young growing pigs (15 to 20 kg BW). Of the most essential AA (Lys, Met, Thr and Trp), AID of tryptophan for the pea diet seems to be underestimated by about 10 percentage units compared with the just above-mentioned literature sources. The AID of CP and AA for the mSBM diet were markedly lower than those for the diet with a commercial SBM. This can be explained by a considerably higher TIA in the mSBM diet compared with the SBM diet (Table 3). Soya trypsin inhibitors (TI) reduce AID of CP and AA in pigs (Vandergrift et al., 1983; Herkelman et al., 1992; Schulze, 1994). The lower AID of CP for the mSBM diet was associated with a significantly higher ENL compared with the SBM diet. This effect is due to the ability of soya TI to form fixed, inactive complexes with proteolytic enzymes of the pancreas and may lead to considerable losses of sulphuric AA (Liener and Kakade, 1980). On the other hand, probably, because of inhibition of proteolytic enzymes by TI (Liener and Kakade, 1980), also the true ileal digestibility of CP in the mSBM diet was markedly reduced (Table 5). Studies of Schulze (1994) showed that increased dietary levels of isolated soybean TI reduced AID of CP in pigs by increased losses of both endogenous and dietary N.

The true ileal digestibility of CP for the pea diet was at a similar level as the SBM diet ($P > 0.05$), but the AID of CP was about 6 percentage units lower ($P < 0.05$). These results agreed with those of Huisman et al. (1992) and Grala et al. (1997b). Ileal DM and ileal endogenous N for the pea diet was likely associated with the presence and nature of the pea carbohydrates (Jørgensen and Jensen, 1994; Lien et al., 1994; Leterme et al., 1996). A high true and a low apparent ileal digestibilities of CP in the pea diet showed that ENL were rather caused by the pea fibres than TI, because the TIA level in the pea diet was low (Table 3).

The AID of CP and of all of the AA for the RC diet were markedly lower than for the SBM diet and generally agreed with previously reported results for the same batch of RC (Grala et al., 1998a). The low apparent and true ileal digestibilities of CP in the RC diet may be, probably, explained by the high content of fibre, especially in the hull fraction (about 25-30% of the oil-free meal; Bell, 1984). Grala et al. (1997a) showed that the AID of CP and AA of rapeseed hulls are very low because of a strong binding of N to the fibre fraction (Bjergegaard et al., 1991). This N (non-protein N) in rapeseed hulls is not digestible at the ileal level (Grala et al., 1997a). Adsorptive properties of fibre may also cause a low true ileal digestibility of CP and a high ileal endogenous N losses with the RC diet (Bergner et al., 1981; Howard et al., 1986), Additionally, this may affect losses of intestinal mucin (Mariscal-Landin et al., 1995) and desquamated epithelial cells (Bjergegaard et al., 1991; Jin et al., 1994).

Nitrogen balance

It has been shown that only AA absorbed before the end of the small intestine are of nutritional value for pigs (Żebrowska et al., 1978; Just et al., 1981). Also an optimal pattern of dietary, essential AA (Fuller et al., 1989; Wang and Fuller, 1989), along with a sufficient energy supply (Bikker, 1994), are needed for a maximum utilization of N (AA) for protein deposition. Only in extreme situations, e.g., severely heat-treated feeds, because of advanced Maillard reactions, a post-absorptive availability of AA may be lower than their ileal digestibilities (Batterham, 1992). This is not the case in the present N balance study, with these soybean products and raw peas. Therefore, the hypothesis for this study was that differences in N retention of pigs fed equal, restricted amounts of apparent ID CP and essential AA are influenced by the ENL, induced by different dietary factors (e.g., TI and/or fibre).

Results of previous studies (Grala et al., 1998b; 1997b) suggested that high ENL coincide with a geater urinary N excretion and less utilization of N for retention. These effects could be indicative of higher N losses during increased protein turnover to replace ENL. An imbalance in the pattern of absorbed AA, as induced by ENL, would cause AA oxidation resulting in increased N losses (Benevenga et al., 1993; Moughan, 1995).

Greater urinary N losses and less N retention in pigs fed the pea and mSBM diets were associated with greater ENL for these diets copared with the SBM diet that caused lowest ENL. These results agreed with previous studies (Grala et al., 1998b; 1997b) and seem to confirm the aforementioned hypothesis. The mSBM diet, which caused the greatest ENL, might have increased the energy and AA requirements for maintenance (extra synthesis of endogenous protein; De Lange et al., 1995; Moughan, 1995) at the expense of N for retention in body tissues (Fuller, 1991; Moughan, 1993). Therefore, the N retention in pigs fed mSBM diet was markedly lower ($P < 0.05$) compared with those fed the SBM diet. On the other hand, a greater ($P < 0.05$) ENL for the pea diet did not increase ($P > 0.05$) excretion of urinary N compared with the SBM diet. This indicates that geater ENL are not always reflected in an increased urinary N excretion. A high presence of fermentable carbohydrates in the pea diet could effect a shift of N excretion from urine to faeces (Younes et al., 1995; Lenis et al., 1996; Canh et al., 1997). On the other hand, pea carbohydrates might increase the gut protein turnover (Jin et al., 1994) that could result in a direct uptake of AA by the intestinal gut wall without passing the liver (Alpers, 1972). As a consequence, this protein synthesis will unnecessarily cause N losses in urine (Armentano, 1994).

Increase in the level of apparent ID, essential AA from 85% in the mSBM diet to 95% of the requirements in the mSBMs diet resulted in an increased N retention from 0.82 to 0.85 g/kg$^{0.75}$/day ($P > 0.05$). Thus, these results may suggest that a higher level of ID AA may compensate for the losses of N (AA) during recycling of endogenous proteins. Moreover, a markedly decreased excretion of N in urine supports the earlier suggestion that extra AA supplementation may improve the profile of AA available for protein synthesis. As a result, the AA oxidation will be minimized, urea formation reduced and finally, urinary N excretion decreased. Consequently, the utilization of ID N for N retention increased by 2.7 percentage units for the mSBMs diet compared with the mSBM diet ($P > 0.05$), being only 1.9 percentage units lower than for the SBM diet.

The results of the present study support suggestions of De Lange et al. (1995), Tamminga et al. (1995) and Nyachoti et al. (1997) that enhanced endogenous protein losses, caused by dietary factors such as protease inhibitors, will reduce post-absorptive efficiency of AA utilization for protein deposition.

Conclusions

The results of this study indicate that losses of ileal endogenous nitrogen, caused by protease inhibitor-dependent factors, were associated with losses of urinary nitrogen. This was associated with lower nitrogen retention. Losses of ileal endogenous nitrogen, caused by fibre-dependent factors, reduced nitrogen retention, but were not reflected in a lower urinary nitrogen excretion. Dietary amino acid supplementation compensated the losses of nitrogen from endogenous protein, by reducing urinary excretion and increasing retention of nitrogen. The study showed that losses of endogenous protein, caused by dietary factors, are important for evaluating the nutritional value of feedstuffs.

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CHAPTER 6

FLOW OF ENDOGENOUS AND EXOGENOUS NITROGEN IN DIFFERENT SEGMENTS OF THE SMALL INTESTINE IN PIGS FED DIETS WITH SOYABEAN CONCENTRATE, SOYABEAN MEAL OR RAPESEED CAKE

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Flow of endogenous and exogenous nitrogen in different segments of the small intestine in pigs fed diets with soybean concentrate, soybean meal or rapeseed cake

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ABSTRACT

Six barrows of with an average initial BW 27.5 ± 1.2 kg were used. Each pig was fitted with two cannulas in different segments of the small intestine: pig 1 in the duodenum (C1) and upper jejunum (C2), pig 2 in C2 and the lower jejunum (C3), pig 3 in C2 and C3, pig 4 in C1 and the terminal ileum (C4), pig 5 in C3 and C4, pig 6 in C1 and C4. Pigs were also fitted with one catheter in the *vena jugularis* for blood sampling and with the second one in the *arteria carotis* for a continuous infusion of the ^{15}N-leucine (4.2 mg/kg$^{0.75}$ BW/d). Pigs were used in a crossover design experiment with three treatments and three periods of digesta collection (36 h) and blood sampling (36 h). Maize starch-based diets that contained: soyabean concentrate (SC), a mixture of toasted and untoasted soyabean meal (mSBM) of a high trypsin inhibitor activity, or rapeseed cake (RC) of a high NDF content were used in the experiment. The flow of endogenous and exogenous N along the small intestine and the net reabsorption rate of endogenous N were estimated.

In the C1 digesta of pigs fed on the SC, mSBM and RC diets, total N was 138, 127 and 126% of the ingested N, respectively. Sixteen, 15 and 10% of the ingested N was absorbed up to C1, and 11.1, 11.6 and 9.4 g of endogenous N (Ed-N) per kg DM intake was secreted, respectively. For corresponding diets in the C2 digesta, total N was 126, 117 and 111% of the ingested N. Thirty four, 11 and 31% of the exogenous N (Ex-N) inflow and -32, 1 and -39% of the Ed-N inflow was absorbed. In the C3 digesta, total N was 44, 47 and 43% of the ingested N, while 81, 71 and 71% of the Ex-N inflow and 54, 38 and 49% of the Ed-N inflow was absorbed, respectively. Accordingly, in the C4 digesta total N was 24, 33 and 33% of the ingested N, and 45, 43 and 4% of the Ex-N inflow was absorbed. True N digestibility at the terminal ileum was 94, 88 and 82% for the SC, mSBM and RC diets. The reabsorption rate of endogenous N before the terminal ileum was estimated as 75, 51 and 69%, respectively.

It was concluded that regardless of the dietary protein source, the middle segment of the small intestine is the site of the most intense absorption of endogenous and exogenous N. Reabsorption rather than secretion of endogenous nitrogen along the pig's small intestine seems to be influenced by dietary factors.

Key Words: pigs; endogenous nitrogen; secretion; absorption; flow

Introduction

Secretion and absorption of nitrogenous compounds occur simultaneously along the intestinal tract in the pig. These two processes are influenced by a number of factors such as age and body weight of animals, protease inhibitors and dietary fibres. Dietary (anti)nutritional factors (ANF) may increase endogenous N losses at the terminal ileum and decrease digestibility of dietary protein (Nyachoti et al., 1997). The energy and protein requirements for maintenance may be increased as a result of extra synthesis of endogenous protein to replenish the losses (De Lange et al., 1995) and, therefore, less dietary N can be used for retention (Grala et al., 1997b). In that respect, the extent of recycling of endogenous protein [secretion, reabsorption and (re)synthesis] may have a significant impact on the post-absorptive efficiency of amino acid utilization for protein deposition (Nyachoti et al., 1997).

Huisman et al. (1993) calculated that about 30% of urinary N may originate from N losses, which occur during recycling of endogenous protein. The calculation is based on the 75% reabsorption of endogenous N before the terminal ileum (Souffrant, 1991). In terms of effective pig production it is important to know whether different losses of endogenous N affected by different protein sources are associated with different or similar secretion of endogenous protein and its reabsorption rate up to the terminal ileum.

The objective of this study was to estimate the flow of endogenous and exogenous N along the small intestine of pigs fed semi-purified diets with soyabean concentrate, a mixture of toasted and untoasted soyabean meal, or rapeseed cake. Another aim of this study was to estimate the net reabsorption rate of endogenous N in the small intestine when using different protein sources. The trial was run on cannulated pigs using the ^{15}N-isotope dilution technique.

Materials and Methods

Protein sources

Three protein sources were used in the study: 1. a commercial soyabean concentrate (SC), 2. a mixture of toasted soyabean meal and untoasted soyabean meal (mSBM; ratio of 75:25, respectively), and 3. toasted rapeseed cake (RC). It was assumed that each protein source affects the flow of endogenous N along the small intestine in pigs in a different way. The SC was considered to cause low flow, while mSMB and RC high flow. The RC was prepared by CETIOM-GERDOC (Pessac, France) from a double-low glucosinolate variety of rape oilseeds (*Brassica napus,* L.). A detailed description of the technological processing of the soyabean and rapeseed products has already been presented elsewhere (Grala et al., 1997b). Chemical characteristics of the feedstuffs is given in Table 1.

Table 1. Chemical characteristics of the tests feedstuffs[1] (g/kg DM)

	SC	mSBM[1]	RC
Dry matter	953.0	909.0	947.0
Crude protein	709.3	555.0	356.9
Ether extract	8.4	13.6	122.5
Ash	72.4	73.6	71.8
Crude fibre	29.4	39.9	153.1
NDF	-	75.7	241.8
TIA, mg/g[2]	2.5	14.5	-
Glucosinolates, μm/g[3]	-	-	12.6
Amino acids			
Arginine	47.6	42.7	21.1
Histidine	19.8	15.6	9.2
Isoleucine	33.2	26.9	14.9
Leucine	57.9	44.5	25.8
Lysine	46.5	37.9	18.6
Methionine	10.3	7.7	6.4
Cystine	11.1	8.8	8.1
Phenylalanine	37.1	33.3	12.1
Threonine	27.3	24.8	17.3
Tryptophan	9.2	7.2	4.6
Valine	35.1	30.9	21.1
Alanine	31.2	29.0	16.8
Aspartic acid	84.8	79.8	27.6
Glutamic acid	127.4	108.7	58.1
Glycine	31.5	26.2	19.1
Proline	45.3	69.4	21.8
Serine	35.7	31.9	18.0
Tyrosine	25.7	19.8	11.8

[1] SC is soybean concentrate, mSBM is mixture of toasted soybean meal and untoasted soybean meal (ratio of 75:25, respectively), and RC is toasted rapeseed cake

[2] Trypsin inhibitor activity (TIA) expressed as milligrams of trypsin inhibited per gram of the product's DM

[3] fat-free DM

Animals and housing

The experiment was carried out using six Polish Landrace barrows. Pigs were 12 to 13-wk-old with BW (± SE) of 27.5 kg (± 1.2 kg) upon arrival. They were individually housed in metabolism cages at an ambient room temperature of 22 to 24°C and a relative air humidity of 55%. Pigs were fed a commercial diet for the adaptation period. The experimental procedures were approved by the ethics committee of The Kielanowski Institute of Animal Physiology and Nutrition.

Table 2. Composition of the experimental diets, %

Item	SC diet	mSBM diet	RC diet
Soya concentrate	18.17	-	-
mSBM[1]	-	31.50	-
Rapeseed cake	-	-	46.78
Maize starch	43.605	39.362	27.274
Dextrose	15.00	15.00	15.00
Soya oil	4.10	2.45	4.05
Cellulose	11.00	4.40	-
$CaCO_3$	1.16	1.20	0.75
$Ca(H_2PO_4)_2H_2O$	1.80	1.70	1.35
NaCl	0.30	0.30	0.30
$KHCO_3$	0.82	0.10	0.45
$NaHCO_3$	0.25	0.25	0.25
MgO	0.06	-	-
Cr_2O_3	0.15	0.15	0.15
Co-EDTA	2.50	2.50	2.50
L-lysine HCl	-	-	0.146
DL-methionine	0.071	0.088	-
L-threonine	0.014	-	-
Premix[2]	1.00	1.00	1.00

[1] Mixture of toasted (75%) and untoasted (25%) soybean meal;

[2] The premix supplied per kilogram of feed: 9,000 IU of vitamin A; 1,800 IU of vitamin D_3; 40 mg of vitamin E; 5 mg of riboflavin; 30 mg niacin; 12 mg of d-pantothenic acid; 1,000 mg of choline, 40 µg of vitamin B_{12}; 2 mg of thiamine; 3 mg of pyridoxine; 0.1 mg of biotin; 1 mg of folic acid; 3 mg of vitamin K; 50 mg of ascorbic acid; 72.8 mg of Zn ($ZnSO_4{\cdot}H_2O$); 44 mg of Mn (MnO_2); 80 mg of Fe ($FeSO_4{\cdot}7H_2O$); .525 mg of Co ($CoSO_4{\cdot}5H_2O$); 0.38 mg of K (KI); 0.254 mg of Cu ($CuSO_4{\cdot}5H_2O$); 0.06 mg of Se ($Na_2SeO_3{\cdot}5H_2O$); 40 mg tylosin.

Table 3. The analysed[1] and calculated[2] chemical composition of the experimental diets, % as-fed basis

Item	SC diet	mSBM diet	RC diet
Dry matter[1]	87.14	86.93	87.60
Crude protein (N x 6.25)[1]	11.28	15.05	14.65
Ether extract[1]	3.35	1.79	8.11
Crude fibre[1]	8.23	3.63	5.86
Ash[1]	4.64	4.96	5.67
NDF[1]	11.50	6.09	11.76
TIA (mg/g)[2]	0.40	3.38	ND
Cr[1]	0.142	0.142	0.142
Co[1]	0.242	0.242	0.242
ID CP[2]	10.4	10.4	10.4
ME, MJ/kg[2]	14.4	14.4	14.4

Diets and feeding

The SC, mSBM and RC were the only protein sources in the maize starch-based diets. Diets were balanced for the apparent ileal digestible (ID) crude protein (CP; N x 6.25) and essential amino acids (AA). The contents of ID essential AA (Lys, Thr, Met+Cys, and Trp) were at least 85% of pigs' requirements (CVB, 1994). Apparent ileal digestibility of CP and AA of the diets containing the same ingredients were determined in a previous ^{15}N / ileal digestibility experiment (Grala et al., 1997b). In the present study, Cr_2O_3 was used as a solid-phase marker (1.5 g/kg feed) and Co-EDTA as a liquid-phase marker (5 g/kg feed). The diets were pelleted in low-temperature conditions (< 60°C). The ingredient content and calculated chemical composition of the diets are given in Tables 2 and 3, respectively.

During the experimental periods pigs were given equal meals at 8.00 and 20.00 h. A daily amount of feed offered to each pig was at a level 2.7 times its maintenance requirement for metabolizable energy (ME) (2.7 x 420 kJ ME/kg$^{0.75}$; ARC, 1981). Water was available *ad libitum* from drinking nipples.

Experimental procedures

The scheme of the experiment is presented in Table 4. After 16 days of the adaptation period, each pig was fitted with two cannulas. The cannulas were placed in the small intestine as follows:

- cannula 1: in the duodenum (C1); T-shape, silicon cannula (ϕ 10 mm); placed about 35 cm distal to the pylorus.
- cannula 2: upper jejunum (C2); T-shape, silicon cannula (ϕ 10 mm); placed posterior to C1 at a distance of about 300 cm.
- cannula 3: lower jejunum (C3); T-shape, silicon cannula (ϕ 20 mm); placed anterior to the end of the ileum at a distance of about 250-300 cm.
- cannula 4: at the terminal ileum (C4); PVTC-cannula (van Leeuwen et al., 1991).

Each pig was provided with two cannulas as following:

pig 1:	C2 + C3
pig 2:	C1 + C2
pig 3:	C2 + C3
pig 4:	C1 + C4
pig 5:	C3 + C4
pig 6:	C1 + C4

Table 4. Scheme of the experimental procedures

Items	Preliminary period				Experimental period							
					Labelling period (fore-period of P1)		Period 1 P1	Fore-period of P2	Period 2 P2	Fore-period of P3	Period 3 P3	The end
Day of exp. (day of exp. period)	1–16	17–19	18–31	32–34	35 (0)^1	36–43 (1–8)	44–46 (9–11)	47–54 (12–19)	55–57 (20–22)	58–65 (23–30)	66–68 (31–33)	69 (34)
	Adaptation	Cannulation	Recovery	Catheterization (taking of "0" blood sample)	Start of the [^{15}N]leucine infusion	Adaptation to experimental diets	a) 3 x 12 h digesta collection from distal cannula b) 3 x 12 h digesta collection from first cannula c) 3 x blood sampling 6 h after feeding	Changing of exp. diets	Similarly to P1	Changing of exp. diets	Similarly to P1	

Cannulation = each pig was fitted with two cannulas in different segments of the small intestine: 1) duodenum (C1) and upper jejunum (C2), 2) C2 and lower jejunum (C3), 3) C2 and C3, 4) C1 and terminal ileum (C4), 5) C3 and C4, 6) C1 and C4.

Catheterization = pigs fitted with one catheter in vena jugularis for blood sampling and with the second one in arteria carotis for a continuous infusion of the [^{15}N]leucine

After 13 days of a recovery, pigs were catheterized with one catheter in the *vena jugularis* for blood sampling and the second one in the *arteria carotis* for infusion of the [^{15}N]leucine (99% ^{15}N-enrichment; Euriso-Top, Belgium). The commercial diet was replaced by the experimental diets and a 34-day continuous ^{15}N-leucine infusion started. The daily rate of the [^{15}N]leucine infusion was about 4.2 mg per kg BW per day. [^{15}N]leucine dissolved in a sterile non-pyrogenic, physiological saline solution (NaCl, 9 g/L) was infused at a rate of about 45 mL/d using perfusion pumps (Fr. B. Braun Melsungen AG, Germany).

Pigs were used in a crossover design experiment comprising three dietary treatments and three periods. Such a design allowed obtaining three observations for each cannula on each treatment. Diets with SC, RC and mSBM were fed as follows:

	Period 1	Period 2	Period 3
pig 1 (C2 + C3):	SC	RC	mSBM
pig 2 (C1 + C2):	SC	RC	mSBM
pig 3 (C2 + C3):	mSBM	SC	RC
pig 4 (C1 + C4):	mSBM	SC	RC
pig 5 (C3 + C4):	RC	mSBM	SC
pig 6 (C1 + C4):	RC	mSBM	SC

Digesta collection lasted 72 h. An 8-day adaptation period to the experimental diets preceded each digesta collection. The first period (P1) was started after 9 days of labelling the pigs with the [^{15}N]leucine. Digesta were collected from the distal cannula for the first 36 h (3 x 12 h). Afterwards the distal cannula was closed and digesta were collected from the proximal one for the next 36 h (3 x 12h). The procedure for the digesta collection was as follows:

A. Duodenal digesta (C1):

The duodenal cannula was opened 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h after feeding time. Digesta (90±10 g) collected for about 5-10 min at each time point were weighed and stored at -20°C until freeze-drying.

Within a period, freeze-dried digesta from the 3 x 12 h collections were pooled per pig and time sampling (14 samples).

B. Jejunal digesta (C-2 and C-3).

Spot samples of 80-100 g of digesta were taken every 1 h (3 x 12 h), immediately frozen and stored at -20°C until analysis.

C. Ileal digesta (C-4):

A continuous collection was performed for 3 x 12 h. Plastic bags with digesta were emptied and weighed every 1 h. Samples were stored at -20°C until pooling per 3 x 12 h collection per pig.

Pooled samples of duodenal and jejunal digesta, and a representative sample of ileal digesta were freeze-dried. Pooled duodenal and jejunal digesta from each sampling time per pig over 36 h (14 samples) were pooled again to obtain a representative sample from 12 h collection for each pig.

Blood sampling (2 x 4 ml) begun during the catheterization when a blank sample was taken to determine the background of the ^{15}N-enrichment in the total N of the trichloroacetic acid (TCA)-blood plasma. Then, blood samples were taken 6 h after feeding (midpoint between feedings) during each 12 h digesta collection period. Blood was centrifuged for 10 min at 2600 rpm and then kept frozen until analysis of ^{15}N-enrichment.

Chemical analyses

Nitrogen, DM and ether extract (EE) in the diets and in freeze-dried ileal digesta were analysed according to standard methods (AOAC, 1984). Neutral detergent fibre (NDF) was determined as described by van Soest (1973). Chromium in the diets and ileal digesta was determined according to Kimura and Miller (1957), and cobalt, using an atom spectrophotometry method of Philips Scientific (1988). The soyabean products were analysed for activity of trypsin inhibitors (TIA) according to van Oort et al. (1989). Glucosinolates of RC were determined according to EEC method No. 1864/90 (1990). The ^{15}N-enrichment of ileal digesta, feed, and of the TCA-soluble fraction of blood plasma was determined according to the procedure of Schulze et al. (1995).

Calculations and data analyses

The ratio of ^{15}N-enrichment excess in digesta to that in the TCA-soluble blood plasma, referred to as the dilution factor, was calculated for each site of digesta collection according to the following equation:

$$DF = (^{15}N_{dig} - {}^{15}N_{d(0)}) / (^{15}N_{pl} - {}^{15}N_{pl(0)}) \times 100 \qquad [1]$$

where DF is dilution factor (%), $^{15}N_{dig}$ is ^{15}N-enrichment of digesta (at. %), $^{15}N_{d(0)}$ is the background ^{15}N-enrichment in the diet (at. %), $^{15}N_{pl}$ is ^{15}N-enrichment in the TCA-soluble blood plasma (at. %), $^{15}N_{pl(0)}$ *is* the background ^{15}N-enrichment in the TCA-soluble blood plasma (at. %).

In digesta for the consecutive cannulas, the amount of total N [g/100 g N intake (NI)] was calculated using Cr and Co as indigestible markers according to the following equation:

$$N_{tfl} = (N_{dig} / N_d) \times (M_d / M_{dig}) \times 100 \qquad [2]$$

where N_{tfl} is the amount or flow of N in particular parts of the intestine, N_{dig} is the N content in digesta (%), N_d is the N content of the diet (%), M_d and M_{dig} are the contents (%) of indigestible markers in diet and digesta, respectively. The N flows were separately calculated for each marker.

The endogenous N (g/100 g NI) was estimated from the dilution factor (Equation 1) and the total N (Equation 2):

$$N_{efl} = DF \times N_{tfl} / 100 \qquad [3]$$

where N_{efl} is the amount of endogenous N in digesta from different cannulas (g/100 g NI). The amount of exogenous (dietary) N in digesta was calculated as the difference between the N_{tfl} and N_{efl}.

For the estimation of the N flow (g/kg dry matter intake-DMI) in consecutive segments of the small intestine, the amounts of N per 100 g NI were related to 1000 g of DMI.

The net absorption of endogenous N, and absorption of exogenous N in each intestinal segment (g/kg DMI) were calculated according to the following scheme: a. NI minus N in C1 digesta, b. N in C1 digesta minus N in C2 digesta, c. N in C2 digesta minus N in C3 digesta, d. N in C3 digesta minus N in C4 digesta. The net absorption of total N in the whole upper intestinal tract was also calculated [(total N in C1 - total N in C4 digesta) / total N in C1 x 100]. Additionally, the net absorption of endogenous N (reabsorption rate) in the small intestine was calculated [(N in C1 - N in C4) / N in C1 x 100] for each treatment. Apparent ileal N digestibility was calculated according to the formula: *NI - N in C4 / NI x 100.*

Part of the scheduled observations could not be included into the data set because of dysfunction of cannulas and/or feed refusals. As a consequence, the number of observations (n) obtained for each cannula and treatment were for the SC diet: C1 = 3, C2 = 2, C3 = 2, C4 = 3; for the mSBM diet: C1 = 2, C2 = 1, C3 = 1, C4 = 3; for the RC diet: C1 = 2, C2 = 1, C3 = 1, C4 = 3.

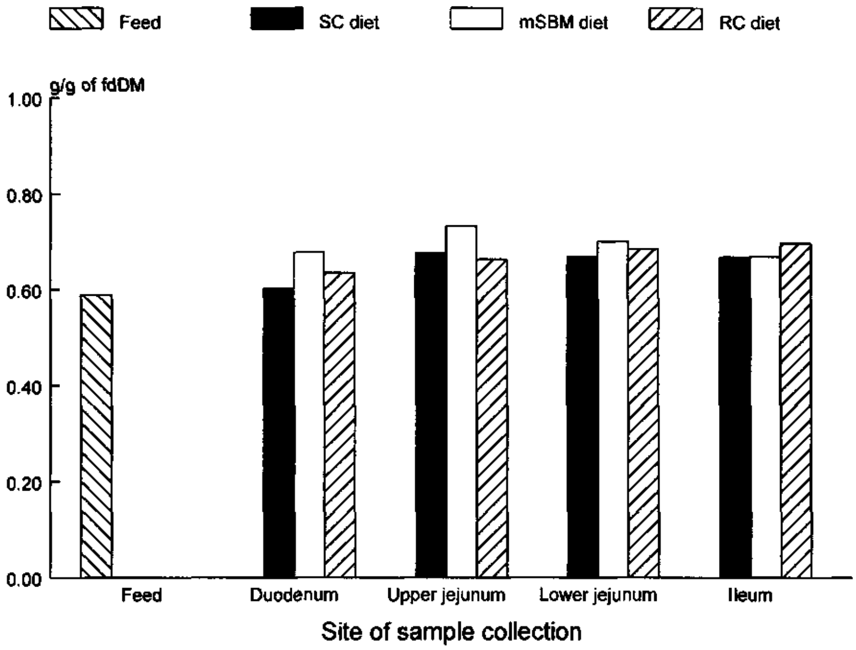

Figure 1. Chromium to cobalt ratios in different segments of the small intestine in pigs fed diets with soybean concentrate (SC), a mixture of toasted and untoasted soybean meal (mSBM), or rapeseed cake (RC)

Results

The flow of both markers (ratio Cr to Co) appeared to be in a constant ratio throughout the different intestinal segments (Figure 1). It was assumed, therefore, that the flow of both solid and liquid phase was constant throughout the upper intestinal tract. The results presented in this section were calculated on the average data for both markers within each treatment.

Table 5. ^{15}N-enrichment excess (at. %)[1,2] in the trichloroacetic acid-soluble blood plasma and in digesta collected from different segments of the small intestine in pigs fed diets with soybean concentrate (SC), a mixture of toasted and untoasted soybean meal (mSBM) or rapeseed cake (RC)

Item	SC diet	mSBM diet	RC diet
Duodenum	[n=3]	[n=2]	[n=2]
Blood plasma[3]		0.0389 ±0.0082	
Digesta	0.0152 ±0.0013	0.0129 ±0.0018	0.0109 ±0.0026
Dilution factor[4], %	39.0 ±0.4	33.1 ±3.1	27.9 ±1.8
Upper jejunum	[n=2]	[n=1]	[n=1]
Blood plasma		0.0381 ±0.0043	
Digesta	0.0215 ±0.0006	0.0135	0.0168
Dilution factor, %	56.3 ±1.4	35.4	43.9
Lower jejunum	[n=2]	[n=1]	[n=1]
Blood plasma		0.0383 ±0.0085	
Digesta	0.0289 ±0.0043	0.0208	0.0221
Dilution factor, %	75.5 ±0.1	54.3	57.7
Terminal ileum	[n=3]	[n=3]	[n=3]
Blood plasma		0.0379 ±0.0093	
Digesta	0.0281 ±0.0025	0.0237 ±0.0009	0.0173 ±0.0029
Dilution factor, %	74.1 ±5.8	62.6 ±2.8	45.6 ±3.3

[1] Values represent means (±SD) when number observations is $n \geq 2$

[2] The background ^{15}N-enrichment of the N in the TCA-soluble blood plasma and feed were 0.3651 and 0.3647, respectively.

[3] Common value for three diets (n = 7 for C1; n = 4 for C2; n = 5 for C3 and n = 9 for C4)

[4] Dilution factor = ^{15}N-enrichment excess in digesta / ^{15}N-enrichment excess in blood plasma x 100

^{15}N-enrichment of blood plasma and digesta

The background ^{15}N-enrichment of the N in the TCA-soluble blood plasma and the diets was 0.3651 and 0.3647, respectively. The mean ^{15}N-enrichment excesses of the N in blood plasma ranged from 0.0379 (±0.0093) at % for C4 to 0.0389 (±0.082) at % for C3 (Table 5). The highest ^{15}N-enrichment excess of the N in digesta was found for the SC and RC diets in C3 while for the mSBM diet, the highest ^{15}N-enrichment excess was in N of digesta from C4.

The highest proportion of endogenous N to total N was estimated in digesta from C3 of pigs fed the SC and RC diet while those fed the mSBM diet had the highest proportion of endogenous N to total N in C4. The lowest dilution factor for each diets was found in duodenal digesta (C1).

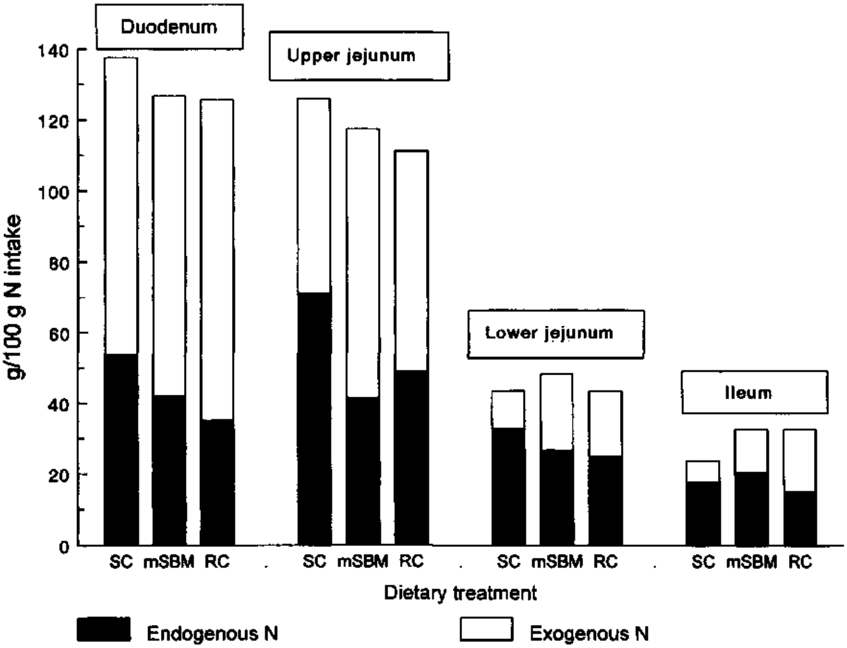

Figure 2. Amounts of total N, endogenous N and exogenous N (g/100 g N intake) in different segments of the small intestine in pigs fed diets with soybean concentrate (SC), a mixture of toasted and untoasted soybean meal (mSBM) or rapeseed cake (RC)

The highest proportion of endogenous N to total N was estimated in digesta from C3 of pigs fed the SC and RC diet while those fed the mSBM diet had the highest proportion of endogenous N to total N in C4. The lowest dilution factor for each diets was found in duodenal digesta (C1).

Nitrogen contents in digesta

The content of total N in duodenal digesta (C1) was 138, 127 and 126 g/100 g NI, for the SC, mSBM and RC diet, respectively (Figure 2). The endogenous N content for the SC and RC diet was higher in digesta from the upper jejunum (C2) than in digesta from C1, but similar to that for the mSBM diet. At the same time, the total N content decreased as compared with the C1 digesta, because the exogenous N content of the C2 digesta was greater by 26, 17 and 11 g/100 g NI, respectively, than the ingested N amounts.

In the more distal segments of the small intestine the amount of total N declined progressively with the greatest difference between C3 and C2. This difference resulted mainly from a decrease in the exogenous N contents. The greatest successive decrease in the contents of endogenous and exogenous N was determined in the digesta of pigs fed the SC diet, while the lowest decrease was found in pigs fed the mSBM diet. Pigs fed the latter diet had a greater content of endogenous N of digesta at the terminal ileum (C4) than those fed the SC and RC diets (21, 18 and 15 g/100 g NI). It was shown that pigs fed the SC diet had the lowest exogenous N content of the C4 digesta, in comparison with pigs fed the mSBM and RC diets (6, 12 and 18 g/100 g NI, respectively).

Nitrogen flow in different segments of the small intestine

Nitrogen flow (g/kg DMI) and absorption rates of N (%) in different intestinal segments are presented in Table 6. In general, the amount of the endogenous N found in duodenal digesta (C1) was similar for all diets (9.4-11.6 g/kg DMI). Ten to 16% of the ingested dietary N (20.7-27.7 g/kg DMI) was already absorbed up to the duodenal cannula. The endogenous N estimated in the upper jejunum digesta (C2) of pigs fed the SC and RC diets was 3.6-3.7 g/kg DMI higher than in duodenal digesta. Pigs fed the mSBM diet, had similar endogenous N flows between the duodenum and upper jejunum. The absorption of exogenous N was 31-34% in the upper jejunum of pigs fed

Table 6. Flow of endogenous and exogenous N (g/kg DMI)[1] in different segments of the small intestine in pigs fed diets with soybean concentrate (SC), a mixture of toasted and untoasted soybean meal (mSBM) or rapeseed cake (RC)

Item	SC diet			mSBM diet			RC diet		
	Endogenous N	Exogenous N	Total N	Endogenous N	Exogenous N	Total N	Endogenous N	Exogenous N	Total N
N intake (NI)	-	20.7	-	-	27.7	-	-	26.8	-
Duodenum		[n=3]			[n=2]			[n=2]	
N secretion	11.1	-	-	11.6	-	-	9.4	-	-
N inflow	11.1	20.7	31.8	11.6	27.7	39.3	9.4	26.8	36.2
N outflow	11.1	17.4	28.5	11.6	23.5	35.1	9.4	24.2	33.6
Net absorption[2]	0	3.3	3.3	0	4.2	4.2	0	2.6	2.6
Absorption NI→C1 [3], %	-	16	10	-	15	11	-	10	7
Upper jejunum		[n=2]			[n=1]			[n=1]	
N inflow	11.1	17.4	28.5	11.6	23.5	35.1	9.4	24.2	33.6
N outflow	14.7	11.4	26.1	11.5	21.0	32.5	13.1	16.7	29.8
Net absorption	-3.6	6.0	2.4	0.1	2.5	2.6	-3.7	7.5	3.8
Absorption C1→C2 [3], %	-32	34	8	1	11	7	-39	31	11
Lower jejunum		[n=2]			[n=1]			[n=1]	
N inflow	14.7	11.4	26.1	11.5	21.0	32.5	13.1	16.7	29.8
N outflow	6.8	2.2	9.0	7.1	6.0	13.1	6.7	4.9	11.6
Net absorption	7.9	9.2	17.1	4.4	15.0	19.4	6.4	11.8	18.2
Absorption C2→C3[3], %	54	81	66	38	71	60	49	71	61
Terminal ileum		[n=3]			[n=3]			[n=3]	
N inflow	6.8	2.2	9.0	7.1	6.0	13.1	6.7	4.9	11.6
N outflow	3.7	1.2	4.9	5.7	3.4	9.1	4.0	4.7	8.7
Net absorption	3.1	1.0	4.1	1.4	2.6	4.0	2.7	0.2	2.9
Absorption C3→C4 [3], %	46	45	46	20	43	31	40	4	25
Absorption C1/2→C4[4], %	75	-	83	51	-	74	69	-	74
Digestibility NI→C4[5], %	-	94	76	-	88	67	-	82	67

[1] Values represent means when number observations are $n \geq 2$

[2] Calculated as N inflow - N outflow

[3] For each intestinal segment calculated as Net absorption / N inflow x 100

[4] Absorption of endogenous N calculated as $(C1/2_{N\ outflow} - C4_{N\ outflow}) / C1/2_{N\ outflow} \times 100$; absorption of total N calculated as $(C1_{N\ outflow} - C4_{N\ outflow}) / C1_{N\ outflow} \times 100$

[5] Digestibility of exogenous N (true) and of total N (apparent) was calculated as $(NI - C4_{N\ outflow}) / NI \times 100$

on SC and RS diets, while it was only 11% for pigs on mSBM diet. The absorption of both endogenous and exogenous N in the lower jejunum (C3) was higher for pigs fed the SC diet than for those fed the mSBM and RC diets. In the last segment of the small intestine (lower jejunum → terminal ileum) absorption of endogenous N was similar for the SC and RC diets (40-46% of the inflow) and lower (20% of the inflow) for the mSBM diet. It was shown that the absorption of exogenous N was 43-46% of the inflow for the SC and mSBM diets while only 4% of the inflow for the RC diet.

Net absorption of total N in the small intestine (duodenum → terminal ileum), was 83, 74 and 74% for the SC, mSBM and RC diet, respectively. Apparent ileal N digestibilities were 76, 67 and 67%, respectively. Total absorption of exogenous N, which is equal to the true ileal N digestibility, was 94, 88 and 82% for corresponding diets. The net reabsorption of endogenous N in the small intestine was 75% for the SC diet, 69% for the RC diet (in both diets estimated for the upper jejunum → ileum), and 51% for the mSBM diet (estimated for the duodenum → ileum).

Discussion

Duodenum

The total N exceeded the N intake by 26-27% (6.8-7.4 g N/kg DMI) in the duodenum of pigs fed the mSBM and RC diets and 38% (7.8 g N/kg DMI) in those fed SC diet. Żebrowska et al. (1982, 1992) and Krawielitzki et al. (1996) reported that the amount of total N in the duodenum of pigs fed diets with various protein sources exceeded the intake by 12 to 20% (3.6-6.7 g N/kg DMI). The higher amounts of total N in duodenum compared with N intake were caused by secretion of endogenous N from such sources as saliva, gastric juice, bile and pancreatic juice (Low and Żebrowska, 1989). In absolute values, assuming no reabsorption of endogenous N in the proximal gastro-intestinal tract (GIT), these sources may contribute to a total of about 6 to 13 g of N/d up to the middle duodenum (Souffrant, 1991). In earlier studies with re-entrant cannulated pigs the flow of endogenous N in duodenal digesta, estimated as the difference between the total N passing duodenum and N intake, was about 7 g/d in pigs fed the casein-based diet (Żebrowska and Buraczewska, 1972) to 14 g/d in pigs fed soyabean meal-based diet (Buraczewska et al., 1975).

In the present study, the use of diets with different protein sources did not influence markedly the flow of endogenous N in the duodenal digesta (range of 9.4 to

11.6 g N/kg DMI). It seems, as far as pancreatic protein secretion is concerned, the different dietary N contents did not affect the amount of secreted endogenous N in pigs. These findings are in agreement with the data of Li et al. (1997).

Ten to 16% of the ingested N was already absorbed up to the middle duodenum. Krawielitzki et al. (1996) using the ^{15}N-isotope dilution technique for the labelling of endogenous N, reported that 8.8 g N/kg DMI of endogenous N was secreted and 14% of ingested N was absorbed in the proximal part of GIT in 30-kg pigs fed wheat and meat-bone meal based diet. Also other studies suggest that part of exogenous N may already be absorbed in the stomach and the anterior duodenum (Żebrowska et al., 1983; Low and Żebrowska, 1989).

Upper jejunum

In the consecutive segments of the small intestine, the flow of both endogenous and exogenous N showed considerable variation among treatments. The flow of endogenous N in the upper jejunum tended to be higher than in the duodenum for the SC and RC diets, while it was as high as in the duodenum for the mSBM. Although, the results for the mSBM and RC diets are based on one observation only, they support findings that the amount of endogenous N secreted in the intestinal juices of the upper segment of the small intestine exceeds, or at least equals, the N amount secreted up to the duodenum (Buraczewska, 1979; Simon and Żebrowska 1988; Souffrant, 1991). In this segment (duodenum → upper jejunum) intensive secretion of endogenous N and protein digestion occurs (Low and Żebrowska, 1989) while absorption is limited at this site (Buraczewska, 1981).

The tendency to lower absorption of exogenous N in pigs fed the mSBM diet, compared with the other two treatments, may be an effect of lower protein digestion affected by a high TI activity.

Lower jejunum

The results of the N flow from the upper jejunum to the lower jejunum (middle part) are in agreement with findings of Buraczewska et al. (1975) and Buraczewska (1981) that this segment of the small intestine plays a dominant role in absorption of nitrogenous compounds. Among the tested diets, the highest absorption of exogenous N (about 80%) was estimated for the SC diet that contained highly soluble dietary protein and low TIA. Also, the absorption of endogenous N was the highest for this diet. Nevertheless, the 70-percentage absorption of exogenous N estimated for the high TIA diet (mSBM), may suggest that there was sufficient enzyme activity for protein digestion in this part of the small intestine. According to Partridge et al. (1982), synthesis and secretion of proteolytic enzymes exceeds the amounts needed to digest dietary protein. Yen et al. (1977) and Żebrowska et al. (1985) showed that the use of raw soyabean, or soyabean TI, does not affect the activity of enzymes in pancreatic juice. Schulze et al. (1993) reported a 30-50-percent (not significant) decrease in secretion of trypsin and chymotrypsin in pigs fed a diet with a high inclusion of purified soyabean TI. The absorption of endogenous N was the lowest in pigs fed the mSBM diet. Pigs fed the RC diet had similar endogenous N absorption to those fed the SC diet.

Terminal jejunum and ileum

In general, relative enrichments of N with ^{15}N and the estimated amounts of both exogenous and endogenous N for the ileal digesta were within the range of values previously reported for the same feedstuffs (Grala et al., 1997a,c; Grala et al., 1997b).

In pigs fed the SC and mSBM diets the percentage of absorption of exogenous N from the last part of the small intestine was similar, while pigs fed the RC diet had very low absorption in this segment of the GIT. Previous results showed that the low digestibility of rapeseed protein is caused by very low digestibility of protein (only 26%) in the hull fraction (Grala et al. 1997a; Grala et al., 1997b). Moreover, most of the hull N is of non-protein origin and is strongly bound to the fibrous fraction. This N is hardly digested in the small intestine because digestive enzymes have restricted access to both the cell wall components and the enclosed cell contents. Additionally, rapeseed carbohydrates (hemicellulose, cellulose, pectin) and lignin may adsorb AA released during protein hydrolysis (Bell, 1984). As a result, more AA of both endogenous and exogenous origin pass into the large intestine.

In pigs fed the mSBM diet only 20% of endogenous N was absorbed in the last segment of the small intestine. Such low absorption may be associated with TI that can form stable complexes with the pancreatic enzymes. In this way they are withdrawn from absorption (Liener and Kakade, 1980).

Total digestion and absorption of endogenous and exogenous nitrogen in the small intestine

The true and apparent ileal digestibilities of exogenous N of the diets, with the exception of the SC diet, were within the range of previously reported values for the same feedstuffs (Grala et al., 1997a,c; Grala et al., 1997b).

Net absorption of total N at the terminal ileum was 83, 74 and 74% of intake for the SC, mSBM and RC diets, respectively. The net reabsorption of endogenous N was estimated as 75, 51 and 69% for the corresponding diets. The value estimated for the SC diet is very close to the results (70-79%) reported by other authors for highly digestible protein sources with low levels of ANF (Souffrant et al., 1986; 1993; Żebrowska et al., 1992; Krawielitzki et al., 1996). Since the other two values from the present study are below the range~~,~~ given above, this may indicate a specific effect of protein sources on the reabsorption rate of endogenous N in the small intestine.

The results of the present study suggest that soyabean concentrate, a mixture of toasted and untoasted soyabean meal, and rapeseed cake cause a different reabsorption of endogenous N up to the end of the small intestine.

Conclusions

Regardless of the dietary protein source fed to pigs, the middle segment of the small intestine is the most intense site of endogenous and exogenous N absorption in the pig. The flow of both endogenous and exogenous nitrogen along the small intestine depended on the protein source. It seems that reabsorption rather than secretion of endogenous nitrogen along the small intestine of the pig seems to be influenced by different dietary factors.

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GENERAL DISCUSSION

General Discussion

Introduction

An efficient animal production, which minimizes harmful effects on the environment, requires feeding strategies that can give an optimal productivity, on the one hand, and limited losses of ingested N, on the other hand (Huisman et al., 1993; Tamminga and Verstegen, 1991). It is generally accepted that in pig nutrition, apparent ileal digestibilities of amino acids provide better estimates of their nutritional availabilities than the faecal ones (Sauer and Ozimek, 1986). However, for particular heat-treated feeds/feedstuffs, post-absorptive availabilities of amino acids may be lower than their apparent ileal digestibilities because amino acids may form unavailable complexes (e.g., Amadori compounds) with other feed constituents (Hurrell, 1990; Batterham, 1992). In any case, amino acids that disappear in the large intestine are not of nutritional significance (Żebrowska et al., 1978), because they are not used for protein synthesis by the pig (Krawielitzki et al., 1983). In the hindgut, especially microbial degradation of both dietary and endogenous protein takes place, which results in formation, e.g., of ammonia, its absorption and conversion to urea, and finally excretion of that in the urine (Low and Żebrowska. 1989).

A number of studies (Low, 1982; Souffrant, 1986, 1993; Żebrowska et al., 1992; Krawielitzki et al., 1994) showed that only about 25% of the total endogenous gut N secretions up to the distal duodenum are recovered at the terminal ileum of pigs. So, the total secretion of endogenous N may be four times higher than estimated at the end of ileum. As a result, increased N losses can be expected during recycling of endogenous N, because protein metabolism has a basic inefficiency (Simon, 1989). In this context, recycling includes protein secretion, its re-digestion and amino acid re-absorption, and then re-synthesis of endogenous protein. In this way, the N losses from endogenous sources may be underestimated to a large extent, if this 75-percentage reabsorption is neglected. From this assumption, and also assuming an equal inefficiency (30%) of protein syntheses for endogenous secretion and body retention, Huisman et al. (1993) estimated that considerable amounts of urinary N losses may originate from the reutilized endogenous N.

At present, therefore, estimates of true digestibilities of protein and amino acids at the ileal level, thus values corrected for the presence of the endogenous protein (N), became important for the correct evaluation of nutritional value of feedstuffs for pigs (Moughan, 1995). In this context, it is essential to know whether a low apparent ileal digestibility of protein is caused by a lower dietary protein digestibility or to a higher excretion of not reabsorbed endogenous protein. With this respect, it is important to estimate ileal endogenous nitrogen losses (ENL) and their metabolic costs associated with N losses in urine. Such an estimation could find ways to reduce N losses and contribute to the reduction of N pollution (Tamminga et al., 1995). Apart from theoretical estimations (Huisman et al., 1993), the possibility of minimizing the environmental N losses by decreasing the endogenous N loss has not yet been studied experimentally.

Starting Hypothesis

The hypothesis for this thesis was based on the assumption that greater ENL are associated with greater secretion of total endogenous proteins. Further, this greater secretion will result in a greater amount of reabsorbed endogenous N (amino acids). As a result, there will be greater amino acid (N) demands for the recycled endogenous proteins (N). Consequently, an increased degradation of amino acids occurs, because of some basic "inefficiencies" of metabolic processes. This degradation will then result in excretion of the N-containing metabolites in urine. Therefore, it was hypothesized that increased ENL may not only lead to extra N losses at the terminal ileum and in faeces, but also in extra N losses in urine. Furthermore, the increased ENL will be associated with a decreased utilization of ileally absorbed dietary N for retention in the pig. This hypothesis holds true when pigs will be fed on a restricted levels of protein and first-limiting amino acids.

Main Findings

The results of this thesis showed that various protein sources (feedstuffs) have different values for apparent and true ileal digestibilities of protein and thus also of ENL (Chapters 1, 2, 4, and 5). In Chapter 1, when using feedstuffs that led to a distinctly different ENL in pigs (wheat gluten - low ENL; peas and rapeseed meal - high ENL), the results on the dietary N utilization for retention were in agreement with the tested hypothesis.

In Chapter 3, it was found that feedstuffs with protease inhibitor-related ENL reduced the dietary N utilization and increased urinary N loss. For feedstuffs with various ENL related to fibre *per se*, the urinary N loss and dietary N utilization for retention did not differ significantly. It means that ENL and N metabolism differ depending on the feed factors to which the ENL are related.

The results of Chapter 3 indicated that the assumed total secretion of endogenous N may not be linearly related to the level of ENL. Therefore, the reabsorption ratio of the secreted endogenous N may vary for different feedstuffs, depending on the specific dietary factors. Based on that, the hypothesis was modified to:

> mainly, the magnitude of ENL (i.e., first-limiting amino acids) determines the inefficiency of dietary N utilization rather than the magnitude of total recycled endogenous N. Thus, increased urinary N losses may depend on the extent of amino acid oxidation resulting from the amino acid imbalance caused by the ENL.

In the particular experimental conditions of Chapter 5 (restricted intake of protein and amino acids), extra supplementation with crystalline amino acids of a diet with high protease inhibitor-related ENL, improved the utilization of the dietary N and decreased extra urinary N loss. It seems, therefore, that extra amino acid addition can compensate for the ENL by improving the profile of amino acids available for endogenous protein re-synthesis. As a result, the remaining dietary amino acids are in a better balance for the body protein retention.

The results in Chapter 6 showed that the total amounts of endogenous N secretions were similar for different dietary factors that induce the ENL. In agreement with the adjusted hypothesis, therefore, the magnitude of the ENL rather than the magnitude of total secreted and recycled endogenous N mainly determines the efficiency of dietary N utilization. Supplementation with extra amino acids showed a possibility to decrease environmental N losses, caused by increased ENL, and to increase the efficiency of dietary N utilization in pig production.

Dietary Causes of Differences in Endogenous Nitrogen Losses

In Chapters 1, 2, 4, and 5, various feedstuffs (wheat gluten, different soybean and rapeseed products, and peas) were used to study their nutritional characteristics. It was shown that proteins of these feedstuffs varied in apparent and true ileal digestibilities distinctly. Also, these feedstuffs led to different ENL in pigs. As has already been discussed in the General Introduction and in individual chapters, the nutritional characteristics of feed proteins depend on characteristics of the proteins and dietary factors, such as antinutritional factors (ANF) and/or fibre. In this study, the dietary factors investigated were trypsin inhibitor activity (TIA) in soybean products, the fibre (NDF) content in rapeseed products, and the combined effects of both TIA and NDF content in peas. These specific dietary factors clearly gave extra ENL in pigs that received these isolated dietary components (Schulze, 1994). Due to the practical nature of the present studies, whole feedstuffs were used to investigate their effects on the ENL instead of isolated dietary ANF or fibres. Therefore, with the use of peas (Chapters 1, 4, and 5) and a raw soybean meal (Chapters 2, 4, and 5), the presence of other protease inhibitors, e.g., lectins (Schulze, 1994) and/or antigenic proteins (Lallès et al., 1993), could also have contributed to the ENL. In the case of rapeseed products (Chapter 1, 2, and 4), an associative, extra effect of phytates could also have contributed to the ENL (Bell, 1984) while tannins and/or sinapine seem to have a minor ANF significance (Blair and Reichert, 1984).

The validity of the method and the accuracy of the ENL estimations are not discussed extensively. Aspects of the limitations of the ^{15}N-isotope dilution technique for quantitative ENL estimations have been discussed in Chapter 2. This study aimed to investigate differences among feedstuffs in ENL and their associative effects on N partitioning in the pig.

Metabolic Processes Affecting Amino Acid Utilization

The efficiency of utilization of exogenous and endogenous protein and amino acid for their metabolism depend on animal status (e.g., phase of growth or production, genetic capacity for protein deposition) on the one hand, and the nutritional characteristics of the feed (e.g., energy supply, protein/amino acid digestibilities and availabilities, profile of amino acids, ANF contents), on the other hand. A schematic model of foremost pools (body compartments) and transaction pathways of ingested protein and endogenous protein is shown in Figure 1.

Generally, ingested protein (N) and endogenously secreted proteins are lost from the body if they are not digested by the pig. Some losses also occur during post-absorptive amino acid metabolism (Simon, 1989). To simplify the model of protein metabolism in Figure 1, all amino acids and/or peptides, available for protein synthesis and oxidation, were combined as a common metabolic pool - free amino acid and peptide pool (FAA). The FAA provides reversible transaction pathways to the other protein pools, such as visceral organs and their endogenous protein secretions. The other protein pool contains muscle, bone and hide proteins. The microbial protein was considered a specific component of the protein flow, because the microbial flora may utilize exogenous and endogenous amino acids for synthesis of protein and growth (Fuller, 1991a; Schulze et al., 1994; Caine, 1997).

In this context, the micro flora may act as a protein-sink by fixing amino acids, affecting their requirements of the pig (Torrallardona et al., 1994).

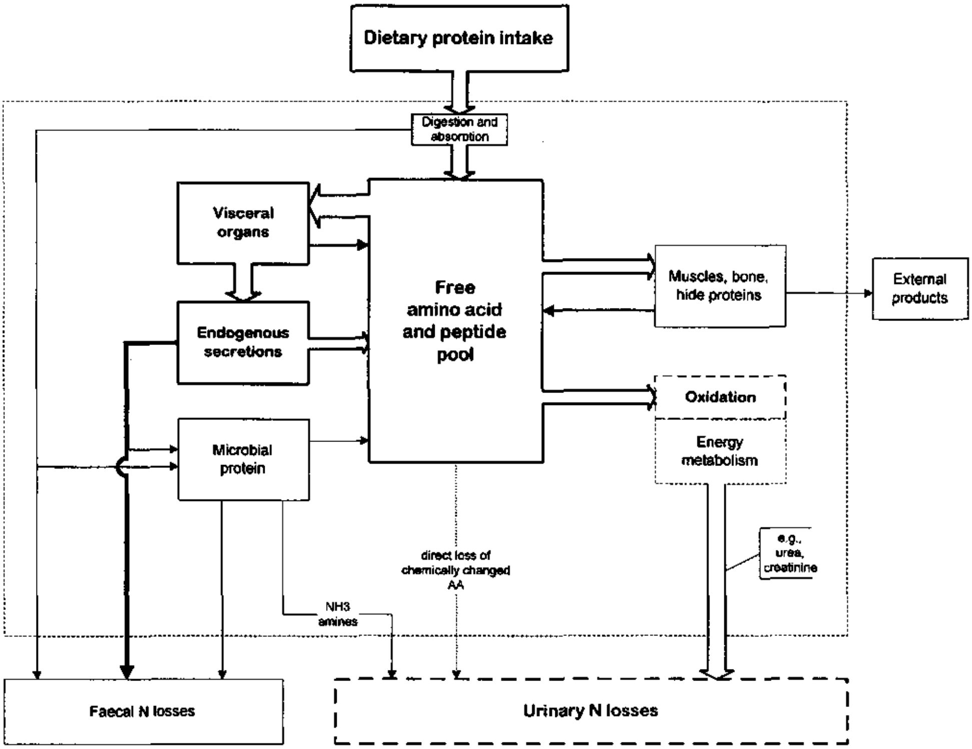

Figure 1. Endogenous and exogenous amino acid and nitrogen fluxes in the pig.

The post-absorptive amino acid metabolism is very complex, highly integrated, and involves several processes, during which amino acids may be degraded and contribute to the urinary N losses. The processes, which can influence the utilization of dietary amino acids, are presented in Table 1.

With regard to the objectives of this thesis, the processes that might affect urinary N losses and the dietary N utilization for N retention are listed in points 1 and 2 of Table 1.

Table 1. Processes contributing to incomplete post-absorptive utilization[a] of the dietary amino acids (after Moughan, 1995)

Physiological processes:

1. Body amino acid maintenance
2. Inevitable amino acid catabolism
3. Preferential catabolism of amino acids for energy supply
4. Absorption of chemically-modified amino acids unavailable for protein synthesis
5. Use amino acids for synthesis of non-protein compounds
6. Trans-amination
7. Catabolism of amino acid supplied above the maximal rate of protein retention

[a] Efficiency of utilization for the net whole-body protein deposition

It seems that maintenance of visceral organs, especially gut, liver and secretory organs, as well as secretion, excretion and replenishment of ENL are of particular importance.

Body Amino Acid Maintenance

As reviewed by Nyachoti et al. (1997), the intestinal gut accounts for a significant amount of the total body energy expenditure. In four months old pigs, oxygen consumption by the gastrointestinal tract is about 25% of total body oxygen consumption. Studies of De Greef (1992) and Bikker (1994) showed that the contribution of viscera to total body weight can vary significantly, and can depend on dietary manipulation.

As outlined earlier, recycling of endogenous protein implies secretion, ENL, digestion/reabsorption and restitution of the ENL to ensure the secretion level (for the pathways, see Figure 1). As a consequence of increased recycling, an increased transport across the gut of metabolites and increased blood flow rate through the tissues can be expected. These processes require energy (Black and De Lange, 1995). Nyachoti et al. (1997) estimated that the maintenance energy requirements may be significantly increased (from 5 to 13% of total energy requirements), when dietary factors, such as protease inhibitors, increase endogenous gut protein turnover as a consequence of increased ENL.

Inevitable Amino Acid Catabolism

The inevitable amino acid catabolism is defined as the loss of amino acids, which have been absorbed, but are not retained in the body and not used for maintenance or for synthesis of non-protein compounds. This amino acid catabolism even relates to amino acids that limit the rate of body protein retention (Moughan, 1995). This phenomenon occurs because there is a basic inefficiency of re-utilization of amino acids during external recycling between body compartments and different tissues as well as during internal recycling within the same cell (Simon, 1989). Estimates for the rate of the inevitable amino acid catabolism can vary largely, and range from negligible to 40% of the absorbed level of the first-limiting amino acid (Moughan, 1995). This large variation may be associated with the level and balance of dietary amino acids fed to the animal (De Lange et al., 1995). This variation may also be related to ENL, when this catabolism applies to the various extent to ENL and gut protein (re)synthesis (Nyachoti et al., 1997). Furthermore, it is likely that the optimum balance of dietary amino acids for body protein retention, is not identical to the amino acid profile of the endogenous proteins (Fuller, 1991b).

Consequences of Endogenous Nitrogen Losses for Efficiency of Nitrogen Utilization

General Functioning of the Hypothesis

Diets with various feedstuffs that resulted in different ENL (wheat gluten, peas and rapeseed) led to variations in the urinary N excretions (corrected for the N of fermentation in the intestinal tract; Chapter 1). The positive relationship between the ENL and extra urinary N losses can be related to the increased losses of reabsorbed endogenous amino acids. As outlined earlier, these amino acids can be oxidized to a large extent and the N-containing metabolites excreted in urine. The use of amino acids for maintenance processes (e.g., replenishment of ENL) have a priority over the use of amino acids for protein deposition (Fuller, 1991b; Moughan, 1993). In this context, the reutilization of endogenous amino acids and ENL may act as an amino-acid-sink by taking up dietary amino acids before they can be used somewhere else. This process probably negatively affected the dietary N utilization for body protein retention in pigs fed the high-ENL diets (peas and rapeseed meal). The results of Chapter 1, generally were in agreement with the starting hypothesis; for the high-ENL diets, the urinary N losses and the inefficiency of the dietary N utilization for retention in pigs were higher than for the low-ENL diet (wheat gluten).

Effects of Endogenous Nitrogen Losses Related to Specific Dietary Factors

A study as to whether ENL from protease inhibitor or fibre containing feeds were similarly associated with a reduced utilization of ileally digested dietary N was described in Chapter 3. Feedstuffs that led to different ENL related to TIA and NDF were used. A clear, positive relationship between the ENL and extra (corrected for fermentation) urinary N excretion was found within the feedstuffs of protease inhibitor-dependent ENL (soybean products). Contrary, a comparison between feedstuffs of fibre-dependent ENL (rapeseed products), showed no relationship between the ENL and extra urinary N excretion. The urinary N excretion, which is discussed here, was corrected for N that could appear in the urine from fermentation in the hind gut. Thus, the latter results were probably not affected by the microbial protein that could increase N excretion in faeces at the expense of that in urine (Younes et al., 1995; Canh, 1997). Moreover, as discussed in Chapter 3, the difference in the faecal N output between the low- and high-fibre diets was directly related to the undigested dietary N. Although toasting had a negative effect on the true ileal protein digestibility (RC1; Chapter 2), the results of N balance eliminated a significant contribution of chemically unavailable amino acids to the urinary N loss and the inefficiency of the dietary N utilization. The utilization of dietary N was not affected by fibre *per se* in the present study and in that of Lenis et al. (1996). Thus, our results did not confirm the hypothesis that the level of reabsorbed endogenous N may determine the N losses when the ENL are related to fibre *per se*.

Nevertheless, the urinary N losses and the utilization of dietary N for retention for the diet with a high, fibre-related ENL (RC2 diet; Chapter 3, Trial II), were generally higher and lower, respectively, than those for the SC diet (Chapter 3, Trial I) that cause a low ENL and contained a minimal levels ANF (TIA).

Reformulation of the Hypothesis

Firstly, there may be a dissimilar physiological response of the pigs' metabolic system to protease inhibitors and fibre. As a consequence, the locations and "length" of metabolic pathways for the reused endogenous protein(s) may be different for these dietary factors.

Secondly, the magnitude of endogenous N secretion may be similar regardless of dietary factors, but is not a fixed, multiplied value of the ENL. As a result, the ratio of endogenous N reabsorption may vary between feedstuffs, depending on dietary factors.

The reuse of the endogenous N may be related mostly to the magnitude of ENL and losses of the specific first-limiting amino acids. Consequently, a significant, irreversible loss of specific endogenous first-limiting amino acids can influence the needs for maintenance amino acids of the pig (Fuller, 1991b). Furthermore, the magnitude of the replenishment of the first-limiting amino acids may be of special importance for the balance of amino acids available in the FAA for protein (re)synthesis. Because the maintenance protein processes have ultimate priority over those for growth (Fuller, 1991b; Moughan, 1993), the dietary amino acids will be used to replenish the ENL, but not for retention. This mechanism may occur when the magnitude of ENL will become critical for metabolic processes, especially on a marginal (restricted) intake of protein and amino acids.

Based on the above, the adjusted hypothesis was:

the reabsorption ratio of total endogenous N secretion to the ENL may vary between the feedstuffs. In this context, the magnitude of the ENL (first-limiting amino acids) may determine the replenishment of these losses by the dietary N (amino acids). Consequently, the magnitude of ENL will determine the inefficiency of dietary N utilization for retention. Furthermore, urinary N losses will depend on the extent of amino acid oxidation resulting from the amino acid imbalance in the FAA (Figure 1) caused by the ENL.

Effects of Dietary Factors on Nitrogen Utilization

Protease inhibitors. Inhibition of proteolytic enzymes can occur (Kakade and Liener, 1980) for feedstuffs which contain high amounts of protease inhibitors. Enzyme inhibition leads not only to a decreased true ileal protein digestibility (e.g., Chapters 3, 4, and 5), but also to the selective loss of specific proteins (i.e., first-limiting amino acids). These losses include the loss of sulphuric amino acids from the pancreatic secretions (Kakade and Liener, 1980). Consequently, the involvement of various visceral organs in the ENL and the replenishment of these losses, as discussed in earlier sections, will require specific amino acids and more energy. These processes will account for higher maintenance protein expenditures in relation to retention protein ones. If the liver is involved in the metabolic pathways, an intensive degradation of amino acids will occur (Armentano, 1994). Consequently, the metabolism of amino acids in different body compartments and tissues can also intensify amino acid catabolism (Simon, 1989). As a result, the N-containing metabolites will add to the urinary N losses (high TIA soybean meal in Chapters 3 and 5).

Fibre. In a presence of rapeseed and/or pea fibres (carbohydrates), a different mechanism for the recycling of endogenous amino acid may occur compared with that of protease inhibitors. As discussed in individual chapters (1, 2, 4, and 5), dietary carbohydrates (e.g., pectines) may increase the gut protein turnover, because of increased desquamation of epithelial cells and production of mucin (Jin et al., 1994). According to Alpers (1972), amino acids for gut protein synthesis can be directly derived from the intestinal lumen without passing the liver. Amino acids are mostly degraded in the liver (Armentano, 1994). Therefore, such a direct uptake of amino acids may to some extent not contribute to extra urinary N losses. A specific effect of fibres on the ENL is an increased excretion of mucoprotein and/or epithelial cells, which are especially rich in threonine (Cetta, 1992; Mosenthin, 1994; Cain, 1997). In this context, restricted threonine transport to the liver may decrease oxidation of this amino acid in the pig (Floc'h et al., 1996). The microbial population can grow in the presence of fermentable fibres (Mosenthin et al., 1994; Schulze, 1994). In that respect, the earlier discussed significant impact of the micro flora on the amino acid requirements of the pig cannot be neglected.

Recycling of the Endogenous Gut Nitrogen

The original hypothesis was based on the assumption that regardless of dietary factors, the secretion of the endogenous N will be four times greater than the estimated ENL (see the Introduction in this chapter). The net reabsorption rate of the secreted endogenous N to the end of the ileum was, however, estimated for diets containing well digested proteins and low in ANF feedstuffs.

Estimations from this study (Chapter 6) showed that the net reabsorption of endogenous N rather than the secretion of endogenous N in pigs is affected by feedstuffs with different dietary factors. For these estimations, the same, selected feedstuffs were used as reported in Chapter 3. The highest net reabsorption of endogenous N was estimated for a soybean concentrate (SC; low ANF and low ENL; 75%). The lowest reabsorption occurred for a soybean meal (mSBM), which had a high TIA and led to a high ENL (51%). The rapeseed cake (RC2 in Chapter 3, but RC in Chapter 6), was used as a source of fibre (NDF) and had an intermediate value (69%). Therefore, it would seem that pigs fed different protein sources, which increase ENL, do not necessarily have an increased magnitude of recycled endogenous N. This conclusion supports findings of Le Guen (1993), Schulze et al. (1993) and Caine (1997) that pancreatic hypertrophy due to the negative feedback regulation induced by trypsin inhibitors does not occur in pigs. Schulze (1994) also suggested that the increased ENL induced by trypsin inhibitors and/or dietary fibre (NDF) is most likely the effect of a decreased reabsorption of endogenous N secretions.

In the light of the modified assumption outlined above, the findings reported in Chapter 6, confirm the negative effect of the magnitude of ENL on the dietary amino acid (N) utilization for retention. These losses will be primarily related to the excessive losses of first-limiting, essential amino acids. So the pattern of amino acid losses will determine the importance of the maintenance needs of the pig. The estimations of Fuller (1991b) showed that exceptionally high requirements are for threonine and sulphur-containing amino acids. The cause and consequences of these amino acids have been discussed earlier.

With respect to the above, a fundamental study is required to estimate the actual magnitude of endogenous protein secretion and its real reabsorption rate to precisely determine the metabolic costs of endogenous protein recycling for the pig, when different protein sources are fed to pigs.

So, as discussed above, the magnitude and profile of losses of the amino acids that supply the FAA (Figure 1), as induced by the ENL (first-limiting amino acids), will in fact determine the inefficiency of dietary amino acid (N) utilization for protein retention. This is due to the fact that maintenance needs (the replenishment of ENL apply to that need) must be fulfilled in an ultimate favour of growth (Fuller, 1991b; Moughan, 1993). Therefore, when continuous supplementation of the FAA with new dietary amino acids is associated with excessive ENL (specific amino acids), the latter will lower the efficiency of amino acid utilization for protein retention. This mechanism can apparently have a large impact on the dietary amino acid supplementation to the FAA, because endogenous N may be (re)digested and (re)absorbed slower than exogenous N (Chapter 6). Additionally, visceral organ functions (i.e., intestines, pancreas) will also be increased to maintain the total endogenous N secretions.

Table 2 does relate to the discussion above, providing some estimates for possible contribution of increased ENL to the increased dietary N requirements for maintenance N. The daily dietary N requirements for the maintenance N was assumed at 0.268 g/kg$^{0.75}$/d (Fuller et al., 1989). It can be calculated that, within the same experimental designs (reported in chapters), the increased ENL may significantly increase the requirements for

Table 2. Estimated impacts of different ileal endogenous N losses (ENL) on the dietary N requirements for the maintenance N in pigs fed various protein sources[a].

Protein source in the diet	Body weight ($kg^{0.75}$)	ENL ($g/kg^{0.75}/d$)	ENL / [ENL +RNM[b]] (%)
Wheat gluten	10.6	0.11	28
Peas batch 1	10.4	0.25	49
Rapeseed meal	10.4	0.25	49
Soybean concentrate	11.6	0.15	36
Soybean meal batch 1	12.5	0.17	38
mSBM batch 1[c]	11.9	0.25	48
Rapeseed cake 1	12.4	0.15	36
Rapeseed cake 2	12.5	0.21	44
Rapeseed cake 3	12.3	0.20	42
Soybean meal batch 2	19.5	0.18	40
mSBM batch 2[c]	19.2	0.33	55
Peas batch 2	19.2	0.24	47

[a] Wheat gluten, peas batch 1, and rapeseed meal related to Chapter 1; soybean concentrate, soybean meal batch 1, mSBM batch 1 (high TIA) relate to Chapters 2 and 3 (Trial I), and rapeseed cakes 1, 2 and 3 relate to Chapters 2 and 3 (Trial II); soybean meal batch 2, mSBM batch 2 (high TIA), and peas batch 2 relate to chapter 5.

[b] RNM = basal dietary nitrogen requirements for maintenance (0.268 $g/kg^{0.75}/d$ [Fuller et al., 1989]).

[c] Mixture of toasted and untoasted soybean meal.

the dietary N needs for the N maintenance compared with the low-ENL feedstuffs. It can be seen that the greater ENL the greater percentage involvement in the maintenance N. The impacts of increased ENL on the greater dietary N requirements for the maintenance N may to some extent explain the lower N utilization for retention. At the same time, possibly, the energetic costs of the replacement of greater ENL can also be increased compared with the low-ENL feedstuffs (Nyachoti et al., 1997).

Furthermore, in the FAA, primarily amino acids available in a proper, balanced pool will only be used for the replenishment of the ileal losses to maintain the endogenous secretions. Subsequently, only amino acids in the FAA that have an exact balance for protein synthesis for body retention can be utilized. The other unbalanced amino acids, supplied in turn in excess, will be oxidized. Consequently, the N-containing metabolites of amino acid degradation will make a substantial contribution to the urinary N losses. As discussed earlier, these losses, which are dependent on ENL, may contribute to the inevitable amino acid catabolism and the inefficiency of endogenous protein synthesis.

In this study, from the truly ileal digestible dietary N available for protein synthsis, the overal efficiency of N utilization for retention and for the replacement of the ENL was about 67% (high TIA and ENL; mSBM diet batch 1, Chapter 2). The highest value was about 85% for the wheat gluten diet, of which the low ENL were associated with a minimal urinary N loss (WG diet, Chapter 1). These estimations include the efficiency of synthesis of endogenous protein, which is associated with the ileal losses, and body protein to be retained.

In this context, it is very difficult to distinguish between the efficiency for endogenous and body protein synthesis. Huisman et al. (1993), for their estimation of N losses from endogenous sources, assumed an equal efficiency of 70%. Sève and Henry (1996), using data of Chapter 1, have estimated the efficiency for endogenous protein synthesis at 55%, and the efficiency for N retention at 88%. In the context of the earlier discussion, such a low efficiency for endogenous protein synthesis is rather questionable. This is due to the fact that the authors have related the efficiency of endogenous protein synthesis only to the N requirements for the replenishment of the ENL from the true ileally N digested N. In their approach, the amount of reabsorbed endogenous N has been omitted.

An important objective for the future research should be the determination of the rate of protein reutilization by the intestinal gut. Then, the studies should focus on the estimation of the rate of endogenous protein (re)synthesis from the reabsorbed endogenous N. Using this approach, it might be possible to estimate the efficiency of endogenous protein synthesis, which is dependent on (anti)nutritive dietary factors.

General Evaluation

As outlined in the General Introduction, and discussed above, only amino acids available for the body protein synthesis are retained. The results of this study imply that apparently digested amino acids are not fully used for N retention. Generally, the extent of ENL and the amino acid profile of these losses determine how inefficiently the dietary amino acids (N) are utilized. Therefore, extra addition of crystalline amino acids will primarily support the replacement of endogenous protein as part of maintenance, improving the profile of amino acids for protein re-synthesis. This addition should result in turn in a greater utilization of amino acids for body retention, because the amino acids remaining in the FAA will be in a better balance. To evaluate this theory, 10% of crystalline amino acids were added to a diet with high ENL and balanced at 85% of requirements for first-limiting amino acid (mSBM; Chapter 5). This addition did not lead to a higher crude protein and energy contents of the diet (mSBMs). The results showed that there is a possibility to compensate for the negative effects of the ENL on the dietary N utilization for retention. A decreased excretion of urinary N (corrected for fermentation) also occurred with the extra amino acid supplementation. These results may support the theory that the amino acid profile available in the FAA for protein synthesis was improved. This improved amino acid profile will result in a lower oxidation of amino acids, of which N-containing metabolites will not add to urinary N losses.

In future research, the possibility to compensate for ENL by the dietary amino acid supplementation should be studied for different levels of amino acid additions, using a range of protein sources containing different (anti)nutritive compounds. This approach might also provide information on the metabolic costs of endogenous amino acid losses in relation to their replenishment. In this way, a more precise nutritional evaluation of feedstuffs that induce ENL can be made for pig nutrition.

General Conclusions

It can be concluded from the previous chapters that:

- various, differently processed protein sources, containing different anti-nutritive compounds, induce different values for apparent and true ileal digestibilities of protein and of ENL.
- Feedstuffs that led to increased ENL caused increased urinary N excretion and decreased utilization of apparent ileal digestible N in pigs (Chapter 1).
- Feedstuffs with protease inhibitor-related ENL potentially increase urinary N loss and reduce the dietary N utilization (Chapter 3).
- The ENL, related to fibre *per se*, may not contribute to the urinary excretion and may affect the utilization of dietary N for retention to a small extent (Chapter 3).
- The magnitude of the ENL rather than the magnitude of total secreted and reabsorbed endogenous N mainly determines the efficiency for dietary N utilization and extra urinary N loss.

- Extra supplementation with crystalline amino acid in the diet, which leads to high protease inhibitor-related ENL, improves the utilization of the dietary N and decreases extra urinary N loss (Chapter 5).
- Extra amino acids with a close profile to the ENL compensate for these losses by improving the profile of reabsorbed endogenous amino acids available for protein synthesis. As a result, there are more available amino acids in a proper balance for body protein retention (Chapter 5).
- The net reabsorption rather than the secretion of endogenous N along the small intestine of the pig is influenced by different dietary factors (Chapter 6).

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SUMMARY

SUMMARY

An intensive animal production is becoming harmful to the environment because of a rather low utilization of the ingested nitrogen (N) for animal protein. In pig production, the ingested N is usually utilized by pigs with efficiency less than 40%. Therefore, N losses must be reduced by improving the output to input ratio of N in pig production to maintain a high productivity without reducing the production volume. Generally, undigested protein in the ileal digesta and faeces comprises both feed protein and non-absorbed endogenous secretions. These N losses can be increased with an increased content of antinutritional factors (ANF) in feeds.

Recently, it has been suggested that there may be a positive relationship between the magnitude of endogenous ileal gut N losses (ENL) and the extent of reabsorbed endogenous N. When the greater ENL will result in greater endogenous N secretion, more endogenous N must be reutilized during metabolic processes. Because these processes are not fully efficient and yield thus N losses, the higher circulation of endogenous caused by ENL may result in extra urinary N losses. Therefore, the endogenous N may to a large extent contribute to the environmental N pollution in pig production.

The objectives of this thesis were to study whether ENL caused by different ANF may lead to extra N losses in urine and can be associated with inefficiency of dietary N utilization for retention in the pig.

The ENL in pigs in all experiments were determined using the ^{15}N-isotope dilution technique ([^{15}N]leucine infused intravenously). To determine the ileal digestibilities of nutrients pigs were fitted with a post-valve-T-caecum cannula (PVTC-cannula).

In Chapter 1, the effect of ileal endogenous nitrogen (N) flow on N balance in pigs was investigated. In two experiments pigs (BW 12 to 24 kg) were fed a maize starch-based diets containing either wheat gluten (WG), peas, or rapeseed meal (RSM). In Exp. 1, apparent and true ileal N digestibilities were 91.4 and 98.6 for the WG diet, 80.5 and 95.6 for the pea diet, 67.2 and 80.1% for the RSM diet, respectively. The ENL ($P < 0.05$) were 2.10, 3.66, and 3.05 g/kg DMI, respectively. In Experiment 2 (N balance), diets were balanced for the contents of apparent ileal digestible (ID) CP (10.8%) and ID essential amino acids. Faecal N digestibility decreased from 92.9 to 82.1 and 72.4% in the WG, pea, and RSM diets, respectively, corresponding to increased urinary N excretion and decreased N retention. The efficiencies of ID CP utilization were 83.5, 79.7, and 75.0%, respectively. The study showed that pigs fed diets with an increased

flow of endogenous ileal N concomitant increase in excretion of urinary N. Nitrogen retention and utilization of ileal digestible N in those pigs were also decreased. In general, the results correspond with the hypothesis for this thesis.

In Chapter 2, the effects of various protein sources on the apparent ileal digestibilities (AID) of CP and amino acids (AA) and on the ENL in growing pigs were investigated. Pigs were fed maize starch-based diets that contained a soy concentrate (SC), soybean meal (SBM), or a mixture of toasted and untoasted soybean meal (mSBM) in Trial I, or three rapeseed cakes, dehulled-toasted (RC1), non-dehulled-toasted (RC2), and dehulled-untoasted (RC3) in Trial II. The protein sources provided diets with similar levels of ID CP (10.8%) and Lys, Met+Cys, Thr, and Trp. The AID of CP was greater ($P < 0.05$) for the SC (86.8%) and SBM (82.8%) than for the mSBM (68.1%) diet. In Trial II, the AID of CP was greater ($P < 0.05$) for RC1 (76.2%) and RC3 (75.8%) than for the RC2 (69.5%) diet. The ENL (g/kg DMI) were greater ($P < 0.05$) for the mSBM diet (3.75) than for the SC (2.15) and SBM (2.53) diets in Trial I but were similar ($P > 0.05$; 2.24, 3.03, 2.89 for RC1, RC2, and RC3, respectively) among diets in Trial II. It was concluded that AID of CP of the soybean diets were associated with endogenous and dietary N losses. For these diets, increased ENL and dietary N losses were associated with a higher dietary trypsin inhibitor activity. For the rapeseed diets, dehulling increased AID of CP and AA, due to reduced ENL ($P = 0.08$) and dietary N losses ($P < 0.05$). Toasting of dehulled rapeseed cake did not affect the AID of CP and AA ($P > 0.05$) while reducing the true ileal CP digestibility ($P < 0.05$).

In Chapter 3, N balance was determined in pigs (BW 24 to 30 kg) fed maize starch-based diets that contained the same protein sources as were studied in Chapter 2. The diets were balanced for the content of ID CP (10.8%) and ID of Lys, Thr, Met+Cys, Trp, and Ile. Feeding level was 2.7 times ME for maintenance per kilogram $BW^{.75}$ and restricted to 88% of the requirements for ID Lys as the first-limiting AA. Compared with the SC diet (low ENL), the diets with SBM (medium ENL) and mSBM (high ENL) resulted in a greater ($P < 0.05$) urinary N excretion. Nitrogen retention tended to be less ($P = 0.08$) in pigs fed diets that caused greater ENL. The utilization of ID N for retention in pigs fed the mSBM diet was lower ($P < 0.05$) than for those fed the SC diet. There were no differences in urinary N excretion, N retention, and the utilization of ID N for retention in pigs fed the rapeseed diets of different fibre contents (hulls as the NDF source). It was concluded that, at similar intakes of the first-limiting amino acid, N retention in pigs fed soybeans tended to be reduced by greater ENL as induced by antinutritional factors (e.g., trypsin inhibitors). Rapeseed hulls, as the predominant fibre source, do not affect N retention and the utilization of ID N for retention.

These inconsistent results, in terms of the tested hypothesis, with protease inhibitor- and fibre-related ENL indicated that ENL and N metabolism may differ depending on the various dietary factors. The hypothesis was adjusted as follows: mainly, the magnitude of rather than the magnitude of recycled endogenous N may determine the inefficiency for dietary N utilization.

In Chapter 4, effects of various feed products, containing different dietary factors, on the AID of CP and on the ENL in pigs were investigated. Pigs (BW 12 to 23 kg) were fed maize starch-based diets containing either SC, purified rapeseed hulls (pRH) and SC (SRH diet), SBM, mSBM or peas. The AID of DM for the SC, SRH, SBM, mSBM, and pea diets were 82.1, 74.3, 83.3, 80.0, and 74.9% ($P < 0.05$), respectively. The AID of CP for these diets were 82.4, 67.6, 81.6, 68.0, and 76.9% ($P < 0.05$), respectively. The AID for CP and for the sum of AA in the pRH, calculated by the difference between the SC and SRH diets, were 26% and 41%, respectively. For the SC, SRH, SBM, mSBM, and pea diets, the ENL (g/kg DMI) were 2.79, 3.46, 2.73, 4.89, and 3.29 ($P < 0.05$), respectively, and the dietary N excretions were 1.16, 4.11, 1.64, 4.15, and 2.53 ($P < 0.05$), respectively. For the SC, SBM and mSBM diets, AID of CP and AA were associated with the ENL and dietary N excretions. The ENL were related to trypsin inhibitor activity in these diets. Some properties of pea fibres negatively affected the AID of CP and AA, and the ENL. The low AID of CP and AA in the pRH were caused by a high content of undigested non-protein N in the fibre fraction.

In Chapter 5, the ENL and N balance in growing pigs (BW 40 to 60 kg) were investigated in two experiments. In Exp. 1, PVTC-cannulated pigs were fed diets based on maize starch with SBM, mSBM, peas, or RC2. The AID of CP for the SBM, mSBM, pea and RC2 diets were 82.8, 72.0, 76.7, and 68.7% ($P < 0.05$). True ileal CP digestibilities for these diets were 96.5, 93.0, 94.0, and 87.5% ($P < 0.05$), and the ENL were 3.08, 6.01, 4.55, and 5.36 g/kg DMI ($P < 0.05$). In Experiment 2 (N balance), the diets contained either SBM, mSBM or peas and were balanced for the contents of ID CP (96 g/kg) and ID essential AA (at least 85% of requirements). The fourth treatment was the mSBM diet, supplemented with essential AA to the level of 95% of the requirements, but contained a similar amount of ID CP (mSBMs diet). For the SBM, mSBM, pea, and mSBMs diets, urinary N excretions were 0.39, 0.59, 0.40, and 0.53 g/kg$^{0.75}$/d ($P < 0.05$), respectively, and N retention was 0.87, 0.80, 0.85, and 0.84

g/kg$^{0.75}$/d ($P < 0.05$), respectively. Utilization of dietary ID N for retention were 79.8, 73.3, 78.2, and 77.6% ($P < 0.05$), respectively. The study showed that higher ENL are associated with higher urinary N losses and with a lower N retention. Supplementation by extra AA of the diet with high ENL may result in a decreased urinary N excretion and an increased utilization of dietary N for retention in pigs.

In Chapter 6, the flow of endogenous and exogenous N along the small intestine and the net reabsorption rate of endogenous N were estimated. Six barrows (initial BW 27.5 ± 1.2 kg) were fitted with two cannulas in different segments of the small intestine: 1) duodenum (C1) and upper jejunum (C2), 2) C2 and lower jejunum (C3), 3) C2 and C3, 4) C1 and terminal ileum (C4), 5) C3 and C4, 6) C1 and C4. Pigs were also fitted with one catheter in vena jugularis for blood sampling and with the second one in arteria carotis for a continuous infusion of the ^{15}N-leucine. Maize starch-based diets that contained SC, mSBM, or RC2 were used in the experiment. In the C1 digesta of pigs fed on the SC, mSBM and RC diets, total N were 138, 127 and 126% of the ingested N, respectively, and 11.1, 11.6, and 9.4 g of endogenous N (Ed-N) per kg DMI was secreted. For corresponding diets in the C2 digesta, total N were 126, 117 and 111% of the ingested N. The outflow of the En-N from the C2 was 32, -1, and 39% higher the Ed-N inflow from the C1. In the C3 digesta, 81, 71, and 71% of the Ex-N inflow and 54, 38, and 49% of the Ed-N inflow was absorbed, respectively. In the C4 digesta the total N was 24, 33, and 33% of the ingested N; 45, 43, and 4% of the Ex-N inflow and 46, 20, and 40% of the Ed-N inflow was absorbed, respectively. True N digestibilities at the terminal ileum were 94, 88, and 82%, respectively. The reabsorption rate of the endogenous N before the terminal ileum was estimated as 75, 51 and 69%, respectively. It was concluded that regardless the dietary protein source, the middle segment of the small intestine is the site of the most intense absorption of endogenous and exogenous N. The reabsorption rather than secretion of endogenous nitrogen along the pig's small intestine seems to be influenced by dietary factors.

In conclusion, various, differently processed protein sources, containing different anti-nutritive compounds, induce different values for apparent and true ileal digestibilities of protein and of ENL. Feedstuffs that lead to increased ENL may decreased utilization of apparent ileal digestible N for retention in pigs. Feedstuffs with protease inhibitor-related ENL potentially increase urinary N loss and reduce the dietary N utilization for retention. The ENL, related to fibre *per se*, may not contribute to the urinary N excretion and may affect the utilization of dietary N to a small extent. The net reabsorption rather than the secretion of endogenous N along the small intestine of the

pig is influenced by different dietary factors. Therefore, mainly the magnitude of the ENL rather than the magnitude of total secreted and reabsorbed endogenous N determines the efficiency for dietary N utilization and extra urinary N loss. Extra supplementation with crystalline AA of diets, which lead to high, protease inhibitor-related ENL, improve the utilization of the dietary N and decreases extra urinary N loss.

The results have shown a possibility for reduction of environmental N pollution from endogenous N sources in pig production.

SAMENVATTING

Samenvatting

De intensieve veehouderij draagt bij aan de belasting van het milieu onder andere als gevolg van een relatief lage benutting van stikstof (N) uit het voer voor eiwitaanzet in het dier. De benutting van via het voer opgenomen N voor aanzet in de vorm van vlees bij varkens is vaak minder dan 40%. Mede om deze reden dient de N-efficiëntie in de varkenshouderij te worden verbeterd zonder dat dit ten koste gaat van de produktiviteit en rentabiliteit. In het algemeen geldt dat niet verteerde N in darminhoud op het einde van de dunne darm bestaat uit zowel niet verteerd N afkomstig uit het voer als uit niet verteerd endogeen N. Endogeen N wordt door het dier aan de spijsbrij toegevoegd tijdens de verteringsgang b.v. in de vorm van verteringsenzymen, darmslijm en afgestorven darmwandcellen. De endogene N verliezen worden onder andere beïnvloed door zogenaamde antinutritionele faktoren (ANF's) in het voer.

Er is recent gesuggereerd dat er mogelijk een relatie bestaat tussen de uitscheiding aan endogeen N op het einde van de dunne darm (ENL) en de totale hoeveelheid endogeen N die voor het einde van de dunne darm wordt verteerd. Op basis hiervan kan worden verondersteld dat naarmate er meer endogeen N op het einde van de dunne darm wordt aangetroffen er ook meer endogeen N in een eerder stadium is (her)verteerd. Vertering en hersynthese van endogeen eiwit gaat gepaard met metabole N-verliezen, die kunnen resulteren in een toename van de uitscheiding van N via de urine. Langs deze weg kunnen (voer)faktoren die de uitscheiding van endogeen N op het einde van de dunne darm van het varken stimuleren dus ook de uitscheiding van N in het milieu vergroten.

De doelstelling van het onderzoek beschreven in dit proefschrift was de relatie tussen de endogene N-verliezen op het einde van de dunne darm van het varken (ENL) en de N-verliezen van N via de urine en de benutting van N voor aanzet in lichaamsweefsel (eiwit/vlees) te onderzoeken.

ENL werden in het onderzoek gemeten met de ^{15}N verdunningstechniek, waarbij ^{15}N leucine intraveneus wordt geïnfuseerd. Doel van het gebruik van ^{15}N is om lichaamseiwit en endogeen eiwit te markeren. De darmverteerbaarheid van eiwit en aminozuren werd vastgesteld met behulp van dieren die waren voorzien van een PVTC canule op het einde van de dunne darm.

In Hoofdstuk 1 is onderzoek beschreven naar het effekt van ENL, geïnduceerd door voeders met verschillende eiwitbronnen, op de N-retentie van biggen (LW 12-24 kg). De drie proefrantsoenen bevatten tarwegluten (WG), erwten of raapzaadschroot (RSM) als enige eiwitbronnen. De schijnbare en ware darmverteerbaarheid voor N bedroegen respektievelijk 91.4, 98.6 % voor het WG rantsoen, 80.5 en 95.6% voor het rantsoen met erwten en 67.2 en 80.1% voor het rantsoen met RSM. ENL waarden bedroegen 2.10, 3.66 en 3.05 g N per kg ds opname. In aansluitend N-balansonderzoek met rantsoenen met een gelijk gehalte schijnbaar darmverteerbaar ruw eiwit (108 g/kg) en darmverteerbare essentiële aminozuren werd een faecale N-verteerbaarheid voor de rantsoenen vastgesteld van respektievelijk 92.9, 82.1 en 72.4%. De N-uitscheiding in de urine nam toe en de N-retentie van de dieren daalde bij gebruik van de drie respectievelijke rantsoenen. De benutting van schijnbaar darmverteerbaar N voor N-retentie bedroeg respektievelijk 83.5, 79.7 en 75.0% voor de drie groepen. Dit onderzoek liet zien dat er een relatie lijkt te bestaan tussen ENL en de benutting van schijnbaar darmverteerbaar N voor N-retentie, hetgeen in overeenstemming is met de hypothese geformuleerd bij aanvang van het onderzoek.

In Hoofdstuk 2 staan de resultaten beschreven van onderzoek naar de effekten van het gebruik van verschillende eiwitbronnen op de schijnbare ileale verteerbaarheid en de ENL bij biggen (LW 9-20 kg). De dieren werden gevoerd met op maiszetmeel gebaseerde rantsoenen met sojaconcentraat (SC), sojaschroot (SBM) en een mengsel van getoaste en ongetoaste sojaschroot (mSBM) als enige eiwitbronnen (onderdeel 1) of drie varianten raapzaadschilfers, ontschild-getoast (RC1), niet-ontschild, getoast (RC-2) en ontschild, niet getoast (RC3) (onderdeel 2). De schijnbare ileale verteringscoëfficiënten voor RE bedroegen respectievelijk 86.8, 82.8, 68.1% voor de rantsoenen met SC, SBM en mSBM en 76.2, 69.5 en 75.8% en voor de rantsoenen met RC1, RC2 en RC3. ENL (g N/kg drogestof opname) was hoger voor het rantsoen met mSBM (3.75) dan voor het rantsoen met SC (2.15) en SBM (2.53) ($P<0.05$) maar waren niet verschillend voor de rantsoenen met raapzaadschilfers (2.24, 3.03 en 2.89 g N/kg ds opname voor RC1, RC2 en RC3). Uit de resultaten voor de op soja gebaseerde rantsoenen werd geconcludeerd dat de schijnbare ileale verteringscoëfficiënten voor RE worden beïnvloed door zowel de ware VC-RE als door ENL. In dit verband lijkt onder andere de trypsineremmeraktiviteit in sojaprodukten een belangrijke faktor. Voor de raapzaadschilfers geldt dat ontschillen de VC-RE en VC-AZ verhoogde, door een sterke tendens tot een verlaging van de ENL ($P=0.08$) en een verhoging van de ware VC-RE ($P<0.05$). Het toasten van ontschilde raapzaadschilfers had geen signifikante invloed op de VC-RE en VC-AZ maar verlaagde de ware VC-RE ($P<0.05$).

In Hoofdstuk 3 is N-balansonderzoek beschreven met biggen (LW 24-30 kg) met op maiszetmeel gebaseerde rantsoenen met dezelfde eiwitbronnen als gebruikt in het onderzoek beschreven in Hoofdstuk 2. De rantsoenen waren gebalanceerd op gelijke gehalten ileaal verteerbaar RE (108 g/kg) en darmverteerbaar lysine, methionine &

cystine, threonine, tryptofaan en isoleucine op een nivo van 88% van de behoefte. Het voernivo bedroeg 2.7 maal de onderhoudsbehoefte voor energie. In vergelijking met het SC rantsoen (laag ENL), resulteerden de rantsoenen met SBM (medium SBM) en mSBM (hoog ENL) tot grotere N-verliezen via de urine ($P<0.05$). De N-retentie tendeerde naar lagere waarden voor beide laatstgenoemde groepen ($P=0.08$). De benutting van schijnbaar darmverteerbaar N (RE) voor N-retentie was lager voor de dieren gevoerd met het rantsoen met mSBM dan voor het rantsoen met SC als eiwitbron. Er werden geen verschillen gevonden in het onderzoek tussen de drie op raapzaadschilfers gebaseerde rantsoenen. Er werd geconcludeerd dat, bij een beperkte opname van RE en limiterende essentiële aminozuren, de N-retentie wordt verlaagd samenhangend met een verhoogde ENL. Dit wordt waarschijnlijk geïnduceerd door antinutritionele faktoren zoals trypsineremmers in soja. Daarentegen hebben celstofrijke raapzaadschillen geen invloed op de N-retentie en benutting van ileaal verteerbaar N voor N-retentie.

Laatstgenoemde resultaten zijn niet consistent met de bij de aanvang van het onderzoek geformuleerde hypothese. Deze resultaten suggereren dat trypsineremmer- en celstof geïnduceerde ENL op een verschillende wijze het N-metabolisme beïnvloeden. Op basis hiervan werd de hypothese geherformuleerd: er werd nu gesteld dat de grootte van de endogene N-verliezen op het einde van de dunne darm (ENL) belangrijker zijn dan de hoeveelheid verteerd (gereabsorbeerd) endogeen eiwit ten aanzien van de benutting van N voor N-retentie.

In Hoofdstuk 4 is voor een vijftal rantsoenen met verschillende eiwitbronnen (SC met en zonder toegevoegde raapzaadschillen, SBM, mSBM en erwten) bij biggen (LW 12-23 kg) de VC-RE en ENL vastgesteld. De VC-RE van deze rantsoenen bedroeg respektievelijk 82.4, 67.6, 81.6, 68.0 en 76.9% ($P<0.05$). De VC-RE en VC-AA van raapzaadschillen werd berekend op respektievelijk 26 en 41%. Voor de rantsoenen bedroeg de ENL 2.79, 3.46, 2.73, 4.89 en 3.29 g N/kg ds opname ($P<0.05$) en de ileale verliezen aan niet verteerd voer-N 1.16, 4.11, 1.64, 4.15 en 2.53 g N/kg ds opname ($P<0.05$). Voor de op soja gebaseerde rantsoenen gold dat de waarden voor VC-RE en VC-AZ werden beïnvloed door zowel verschillen in ENL en als door verschillen in de hoeveelheid niet-verteerde voer-N op het einde van de dunne darm. ENL was gerelateerd aan de trypsineremmeraktiviteit in de rantsoenen. De lage VC-RE en VC-AA van de raapzaadschilfers werd veroorzaakt doör het hoge gehalte aan niet-eiwit stikstof (NPN) in de celstoffraktie.

In Hoofdstuk 5 zijn de ENL en N-retentie van groeiende varkens (LW 40-60 kg) vastgesteld in een tweetal experimenten. In het eerste experiment werden rantsoenen met SBM, mSBM, erwten en RC2 als eiwitbronnen onderzocht. De VC-RE van deze rantsoenen bedroeg respektievelijk 82.8, 72.0, 76.7 en 68.7% ($P<0.05$). De ware VC-RE voor deze rantsoenen bedroeg 96.5, 93.0, 94.0 en 87.5% ($P<0.05$) en de ENL 3.08, 6.01, 4.55, en 5.36 g N/kg ds opname ($P<0.05$). In het N-balansonderzoek werden een drietal rantsoenen onderzocht met SBM, mSBM en erwten als eiwitbronnen en 96 g/kg darmverteerbaar RE en essentiële aminozuren op een nivo van minimaal 85% van de behoefte. Een vierde variant bestond uit een rantsoen met mSBM als eiwitbron en 96 g/kg darmverteerbaar RE maar gesupplementeerd met vrije aminozuren tot een nivo van 95% van de behoefte (mSBMs). Bij gebruik van de vier respectievelijke rantsoenen bedroeg de N-uitscheiding in de urine respektievelijk 0.39, 0.59, 0.40 en 0.53 g/kg$^{0.75}$/d ($P<0.05$) en de N-retentie 0.87, 0.80, 0.85 en 0.84 g/kg$^{0.75}$/d ($P<0.05$). De benutting van schijnbaar darmverteerbaar N voor N-retentie bedroeg 79.8, 73.3, 78.2 en 77.6% ($P<0.05$). De resultaten van deze studie lieten zien dat een hogere ENL gepaard gaat met een verhoogde N-uitscheiding via de urine en een verlaagde N-retentie. Supplementering van een rantsoen met een hoge ENL met extra vrije, essentiële aminozuren kan leiden tot een verlaagde N-uitscheiding via de urine en een verbeterde benutting van N in het voer voor N-retentie.

In Hoofdstuk 6 staan de resultaten van onderzoek vermeld naar de uitscheiding en absorptie van endogene en exogene N in verschillende delen van de dunne darm van het varken. Hiertoe werden zes borgen (LW 27 kg) voorzien van een tweetal T-canules op verschillende plaatsen in de dunne darm: dier 1. in het duodenum (C1) en het eerste deel van het jejunum (C2), dier 2. in C2 en in het tweede gedeelte van het jejunum (C3), dier 3. in C2 en C3, dier 4 in C1 en C4, dier 5 in C3 en C4, en dier 6 in C1 en C4. De dieren waren ook voorzien van catheters in de bloedbaan voor de continue infusie van ^{15}N-leucine en voor bloedbemonstering. De drie proefrantsoenen bevatten SC, mSBM en RC2 als enige eiwitbronnen. In het duodenum (C1) bedroeg het N-gehalte van de digesta 138, 127 en 126% van de waarden in de respectievelijke voeders en de endogene N-secretie 11.1, 11.6, en 9.4 g per kg ds opname. In het tweede deel van het jejunum (C2) bedroegen de corresponderende waarden respektievelijk 126, 117 en 111%. De passage van endogeen N bij C2 was 32, -1 en 39% hoger dan bij C1. In het tweede deel van de dunne darm (tussen C2 en C3) werd 81, 71 en 71% van het binnenkomende voer-N verteerd en werd 54, 38 en 49% van het binnenkomende endogene N schijnbaar geabsorbeerd. Op het einde van de dunne darm (C4) bedroeg de totale N 24, 33 en 33% van de N-opname via het voer; 45, 43 en 4% van de passage bij C3 van het niet verteerde voer-N en 46, 20 en 40% van de endogene N passage bij C3 was hier geabsorbeerd. De ware VC-RE voor de drie voeders bedroeg 94, 88 en 82%. De schijnbare herabsorptie van endogeen N tussen het duodenum en het einde van het ileum werd berekend op 75, 51 en 69% voor de drie respectievelijke voeders. Er werd geconcludeerd dat, onafhankelijk van de eiwitbron in het rantsoen, het middengedeelte van de dunne darm de belangrijkste plaats is voor de (re)absorptie van endogeen en exogeen N. Daarnaast suggereren de resultaten dat de herabsorptie van endogeen N in het verteringskanaal sterk wordt beïnvloed door het rantsoen.

Er kan worden geconcludeerd dat rantsoenen met plantaardige eiwitbronnen met verschillende gehalten aan antinutritionele faktoren, zowel de ware verteerbaarheid van het voereiwit als de uitscheiding van endogeen N op het einde van de dunne darm beïnvloeden. Voedermiddelen die aanleiding geven tot een verhoogde uitscheiding aan endogeen N op het einde van de dunne darm van varkens kunnen ook de benutting van N voor N-retentie in negatieve zin beïnvloeden. Voedermiddelen met trypsineremmeraktiviteit, welke ENL stimuleren, kunnen in gebalanceerde rantsoenen de N-uitscheiding via de urine verhogen en de benutting van darmverteerbaar N voor N-retentie verlagen. Een verhoogde uitscheiding van endogeen N veroorzaakt door celstof in het rantsoen heeft veel minder invloed op de N-uitscheiding in de urine en N-benutting. Tevens werd geconcludeerd dat de herabsorptie van endogeen N bij varkens meer wordt beïnvloed door verschillende rantsoenfaktoren dan de secretie. Daarom bepaalt de grootte van endogene N verliezen op het einde van de dunne darm van het varken meer dan de totale hoeveelheid gesynthetiseerde en gereabsorbeerde endogeen N de efficiëntie van het gebruik van N in het voer en de uitscheiding ervan in de urine. Supplementering van een rantsoen met een hoge uitscheiding van endogeen N op het einde van de dunne darm met extra vrije, essentiële aminozuren kan leiden tot een verlaagde N-uitscheiding via de urine en een verbeterde benutting van N in het voer voor N-retentie.

De resultaten van het onderzoek beschreven in dit proefschrift laten zien dat endogene eiwitsynthese en hieruit resulterende N-verliezen een belangrijke invloed hebben op de N-uitscheiding door varkens in het milieu.

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CURRICULUM VITAE

Witold Antoni Grala was born in January 25, 1958 in Wola Zaradzyńska, Poland. In 1983, he received his Masters degree (M.Sc.) from the Department of Animal Production of the Warsaw Agricultural University, Poland,, with a specialization in Animal Nutrition and Production.

From October 1983 to October 1984, he worked at the Central Polyclinic, Warsaw, Poland, in the Laboratory of Experiments and Experimental Animals as a specialist in breeding of laboratory animals.

From November 1984 to March 1986 he was at the Corporation for Trade of Farm Animals (POZH), Sanok, Poland as a specialist in the export and turnover of farm animals.

In April 1986, he joined The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna, Poland. Where he worked until 1991 on the physiology of nutrient digestion in pigs. This involved techniques such as cannulation of the intestinal tract and surgical isolation of the pancreatic duct while studying specifically the effects of commercial processing of rapeseed meal on its protein quality and availability in pig and poultry nutrition.

In April 1992, the Polish Academy of Sciences promoted him to a doctor's degree in agricultural sciences (PhD), after defending his thesis entitled "Biological value of protein of double low rapeseed meal treated at different temperatures" [in Polish].

From September 1991 throughout 1992, and from October 1993 until February 1994, he has taken part in different projects as a visiting research fellow in the TNO-Nutrition and Food Research Institute, Department. for Animal Nutrition and Physiology (ILOB), Wageningen, The Netherlands. For last period, from October 1993, his research programme included studies on ileal endogenous nitrogen losses and their influence on the dietary nitrogen utilization in pigs.

From March 1994 until November 1997, he has been working at the Animal Nutrition Group of the Wageningen Institute of Animal Sciences (WIAS), Wageningen Agricultural University, The Netherlands.