ENDOGENOUS ILEAL NITROGEN LOSSES IN PIGS

- DIETARY FACTORS -

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PROEFSCHRIFT

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Schulze, H., 1994. Endogenous ileal nitrogen losses in pigs, dietary factors (Endogene N verlies in dunne darm van varkens, invloed van voer factoren).

The determination of endogenous protein from the gastro-intestinal tract of the pig is of fundamental importance in nutritional science. It is generally known that various dietary and animal factors may cause additional endogenous protein losses at the terminal ileum in the pig. In this thesis, endogenous ileal nitrogen (N) losses, as affected by a) neutral detergent fibre (NDF) purified from wheat bran, b) trypsin inhibitors (TI) and c) lectins (Le) isolated from soybean, were studied in young growing pigs using the ^{15}N-isotope dilution technique. From methodological studies, it was concluded that the ^{15}N-isotope dilution method is a valid method for determining endogenous ileal N losses in the pig. It was found that the inclusion of purified NDF, isolated TI and Le in the pigs' diet, led to an increased amount of total N passing the terminal ileum of the pig. This increase was caused by additional ileal losses of both endogenous and exogenous N. Even a very small amount of ingested lectins, similar to amounts found in well toasted soybeans, caused an increase in endogenous ileal N loss. The increase in additionally excreted endogenous ileal protein was found to be linear with the amounts of purified NDF and TI. Purified NDF from wheat bran and NDF in sun flower hulls, induced similar endogenous ileal N losses in the pig. The dietary inclusion of whole wheat bran showed that apart from the NDF, there are some other factors, which induced an extra endogenous N loss.

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STELLINGEN

1. Quantification of endogenous ileal protein losses is of practical importance for dietary requirement of essential amino acids. (this thesis)

2. True ileal digestibility can be considered as a fundamental property of the feedstuff protein i.c. the max digestibility of the feedstuff protein for an animal. (this thesis)

3. The ^{15}N-Isotope Dilution Method is a valid method to determine endogenous ileal nitrogen losses in pigs. (this thesis)

4. Measuring the NDF content of a diet is not sufficient enough to estimate the endogenous protein losses at the terminal ileum of pigs affected by dietary fiber. (this thesis)

5. The results of experiments carried out with rats to investigate the effect of trypsin inhibitors are not transferable to pigs. (this thesis)

6. Reduction of trypsin inhibitor and lectin contents in pigs' diet is of very high economical and environmental importance. (this thesis)

7. Endogenous nitrogen is one of the major importance for the low efficiency of protein utilization in monogastric animals.

8. Endogenous ileal protein can be seen as a consequence of the fight of the organism for maintaining his balance.

9. Der finanzielle Wert eines "threshold levels" liegt um einiges über dem einer wissenschaftlich begründeten "dose-response-curve".

10. "Habe nun, ach! Philosophie, Juristerei, Medizin, und leider auch Theologie durchaus studiert, mit heißem Bemühn. Da steh' ich nun, ich armer Tor! Und bin so klug als wie zuvor, ..."

"Faust, Der Tragödie erster Teil" J.W. von Goethe

11. Wenn jeder etwas mehr Verantwortlichkeit und Respekt gegenüber seinen Mitmenschen und der Natur entgegenbringt, werden eine Unzahl von Gesetzen zur Regelung des Zusammenlebens überflüssig.

H. Schulze Endogenous ileal nitrogen losses in pigs: dietary factors.
Wageningen, 29 juli 1994

VOORWOORD

Dit proefschrift is het resultaat van onderzoek uitgevoerd bij de vakgroep Veevoeding van de Landbouwuniversiteit en het Instituut voor Voeding en Fysiologie van Landbouwhuisdieren. Hoewel mijn naam op de omslag van dit proefschrift staat, was het mij zonder de hulp van mijn vrienden, collega's en familie niet gelukt.

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Wageningen, 29 juli 1994 *Hagen Schulze*

CONTENTS

General Introduction

GENERAL INTRODUCTION

To ensure economically viable and environmentally acceptable livestock production, it is essential to provide a well-balanced diet. This diet should support adequate and efficient growth of the animal. Therefore, feedstuffs have to be evaluated and valued for their biologically available nutrients. Besides that, a knowledge and understanding of nutrient requirements and allowances of the animal are required. For intensive livestock production systems such as pig production, protein deposition is of major importance. The production of animal protein involves partitioning of the dietary protein between nitrogen (N) in protein retention, and waste N in faeces and urine. One of the objectives of the nutritionist is to control this nitrogen partitioning. To do this effectively, an evaluation of the feedstuffs with regard to potential protein for retention is of considerable importance.

Historically, the addition of protein to pig diets was based on the crude protein (N x 6.25) content of the ingredients, and later on digestible crude protein. With the development of new analytical methods, feed formulation was based on the amino acid content of the ingredients and later on digestible amino acids. The amino acid composition of a feedstuff protein when determined by chemical methods, does not represent the amount of digested amino acids for the pig (Tanksley and Knabe, 1993). In order to improve and/or optimize diet formulation for pigs, the levels of digestible rather than total crude protein and amino acids in feedstuffs are essential (Furuya and Kaji, 1989). The amount of digestible crude protein and amino acids used to be measured as the amount present in the diet minus that in the faeces. However, it is now generally accepted that the measure of digestibility at the end of the small intestine is a more accurate estimate of amino acids availability, due to the modifying action of the microflora in the large intestine (i.e. Sauer and Ozimek, 1986; Tanksley and Knabe, 1993). Microbial activity in the large intestine comprises both degradation of nitrogenous substrates reaching the large intestine, and synthesis of microbial protein (Rérat, 1978). The nutritional impact of these modifications seems to be limited, as there is almost no amino acid absorption at the large intestine (Zebrowska, 1973). In the current situation in the Netherlands, ileal digestible amino acids are used to formulate feeds for pigs. In this approach, it is assumed that ileal digestible amino acids are fully available to the pig. In a recent study with a slope ratio assay, Batterham et al. (1990) indicated, that lysine ileal apparent digestibility considerably overestimated its availability in pigs, especially in heated products. Availability of an amino acid is defined as the relative amount digested and absorbed and in a form suitable for utilisation. To formulate pigs diets based on available amino acids however, rapid inexpensive techniques for assessing amino acid availability in feeds have to be developed.

It should be recognized, that ileal digesta contains appreciable quantities of non-dietary protein. The classical definition for the excreted protein that does not originate from the feed was given by Mitchell (1924). This protein, also referred to as endogenous protein, is defined as the protein found in chyme or faeces when a N-free diet is fed. Endogenous ileal protein losses are derived from the gastrointestinal tract, and comprise protein, peptides, amino acids and other N-containing substances from saliva, bile, pancreatic, gastric and intestinal secretions, plasma, and sloughed epithelial cells. Bacteria and ingested body hair are also included in the measurement of endogenous loss even though they are not strictly endogenous. By correcting the total amount of ileally excreted protein for this endogenous ileal protein component, the true ileal protein digestibility of ingested feed protein can be estimated. Therefore, the true ileal digestibility of crude protein is a better estimate of dietary protein availability and is a fundamental property of the feed ingredient. Endogenous N is than a consequence of the reaction of the animal to specific properties of the feedstuff.

The excretion of endogenous or non-dietary protein (N x 6.25) leads to an underestimation of the proportion of dietary protein actually absorbed by the animal. Therefore, it is important to have quantitative measurements of endogenous N losses. From these, techniques can be developed to reduce those losses. This is of practical importance for the evaluation and improvement of the feed protein quality and for reducing faecal as well as urinary N excretion in monogastric farm animals (Huisman et al., 1993). The determination of endogenous N is also of importance for the determination of protein and/or amino acid requirements for maintenance (Moughan, 1989). This may be essential for the efficiency of protein gain.

It is important to know the dietary and animal factors responsible for the magnitude of endogenous ileal N losses. Animal factors can include the protein status of the animal (de Lange et al., 1989), age (development of the digestive system), and health status (Moughan, 1993). The dietary factors associated with the variation in endogenous N excretion depends on many factors, including dry matter and protein intake (Krawielitzki et al., 1977; Butts et al., 1993a), protein quality and structure (Gebhardt et al., 1981; Moughan and Rutherfurd, 1990; Butts et al., 1993b), level and composition of crude fibre (Sauer and Ozimek, 1986) and the content and source of antinutritional factors (Huisman, 1990; Jansman, 1993).

Some values for endogenous ileal N excretion which resulted from resent research carried out cooperatively by the Wageningen Agricultural University, together with TNO - Department for Animal Nutrition and Physiology (ILOB) and the Research Institute for Biology of Farm Animals, Department of Nutrition and Physiology "Oskar Kellner" (Germany), are shown in

Table 1. These values were estimated with the ^{15}N-isotope dilution method in young growing pigs (about 10 kg liveweight) which had been fed various feedstuffs. The values clearly show that there is a wide variation in the apparent ileal protein digestibility, and the excretion of endogenous ileal N losses between feedstuffs. True ileal N digestibility was calculated by correcting the apparent ileal N digestibility for losses of endogenous ileal N.

TABLE 1. Apparent and true ileal nitrogen digestibility (%) and endogenous ileal nitrogen losses (g/100 g ofingested N) fed various protein sources to young growing pigs (about 10 kg liveweight).

Protein source	Ileal Digestibility		Endogenous
	Apparent	True	Nitrogen
Skim milk powder[a]	84.4	92.7	8.3
Soybean meal[a]	76.5	90.6	14.1
Fish meal[b]	73.0	89.3	16.3
Soya isolate[c]	78.4	98.4	20.0
Peas (*Pisum sativum var. finale*)[d]	79.0	95.1	16.1
Peas (*Pisum sativum var. frijaune*)[d]	74.1	92.9	18.8
Beans (*Phaseolus vulgaris var. processor*, toasted)[e]	-3.9	65.8	69.7
Beans (*Vicia faba var. Alfred*, high tannins)[f]	74.1	90.5	16.4
Beans (*Vicia faba var. Alfred*, dehulled)[f]	88.2	97.2	9.0
Beans (*Vicia faba var. Toret*, low tannins)[f]	88.7	94.4	5.7

[a] Makkink and Heinz, 1991; [b] Makkink, 1993a; [c] Makkink, 1993b; [d] Huisman et al., 1992; [e] Van der Poel et al., 1991; [f] Jansman, 1993.

The variation in true ileal N digestibility was generally smaller than for the apparent ileal N digestibility. The difference between apparent and true ileal digestibility is caused by the amount of endogenous N still present at the terminal ileum. The higher variability of apparent digestibility was for a major part, due to the variation in endogenous ileal N losses. For their studies on endogenous ileal N losses in piglets Makkink and Heinz (1991) and Makkink (1993a,b) used skim milk powder as a positive control and compared this with other protein rich feedstuffs. It was found by these authors (Table 1) that protein rich feedstuffs such as soya isolate, soybean meal or fish meal, caused an additional ileal excretion of endogenous protein when compared to skim milk powder. Induced by the increased application of legume seeds in feed formulations, endogenous ileal N losses after feeding various varieties of peas and beans and the effect of dehulling were studied (Van der Poel et al., 1991; Huisman et al., 1992; Jansman 1993). It was found that the excretion of endogenous protein in piglets fed feedstuffs

such as soya, fishmeal, peas and beans could be more than twice as high as that in a diet containing proteins such as skim milk powder, dehulled faba beans, or faba beans low in tannins (Table 1). The estimated endogenous losses, however, may be the result of a number of factors operating at the same time. Therefore, specific factors in the diet that affect the losses of endogenous N in ileal digesta need to be known.

Although the need for correction of apparent protein (N x 6.25) digestibility values for endogenous excretion is recognized, there are problems in the estimations of the amount of protein in ileal digesta that is of dietary or endogenous origin. The endogenous flow of protein and amino acids has traditionally been determined after feeding a protein-free diet. Recent studies (Darragh et al., 1990; de Lange et al., 1990; Moughan and Rutherfurd, 1990; Butts et al., 1991, 1993a) have shown that endogenous protein losses from the small intestine, are greater following the feeding of diets containing peptides and protein than under protein-free or synthetic amino acid alimentation. Alternative methods to estimate endogenous protein losses under conditions of protein or peptide feeding are the homoarginine method (Hagemeister and Ebersdobler, 1985), the peptide alimentation ultrafiltration method (Moughan et al., 1990) and the ^{15}N-isotope dilution method. Although the assumptions and validation of the ^{15}N-isotope dilution method are still the subject of scientific discussions (de Lange et al., 1992; Moughan et al., 1992), it has become one of the most widely used methods to determine the endogenous N flow at the distal ileum of pigs (i.e. de Lange et al., 1990; Huisman et al., 1992; Souffrant et al., 1993). In this method, the contribution of endogenous N to total N in the ileal digesta, is calculated from the ratio of ^{15}N enrichment in ileal digesta and in an appropriate precursor pool, assuming that the ^{15}N enrichment of endogenous N is similar to that of the precursor pool.

The objective of this thesis is to investigate the dietary factors that influence endogenous N excretion at the terminal ileum of young growing pigs. Independently isolated fractions were used to study the effects of these dietary factors.
Since the method chosen to measure endogenous N losses at the terminal ileum of pigs form the baseline for the following studies, the initial studies (Chapter 1) concentrate on some *methodological aspects of the ^{15}N-isotope dilution method.* In Chapter 2 the *^{15}N-isotope dilution method was evaluated* by comparing estimated endogenous N losses of this method with the recently introduced peptide alimentation ultrafiltration method.
In Chapter 3 the effect of *isolated wheat bran neutral detergent fibre* on endogenous ileal N excretion in young growing pigs was studied. The effects of different neutral detergent fibre sources on endogenous N excretion in pigs at the terminal ileum, are compared in Chapter 4.

The effect of *isolated soybean trypsin inhibitors* on endogenous ileal N losses in young growing pigs was studied in Chapter 5. Investigations regarding the effect of *isolated soybean lectin* on endogenous ileal N excretion are presented in Chapter 6. Finally, the major findings of the Chapter 1 to 6 are discussed in the General Discussion. Prospects for further research including the methodology used to estimate endogenous N losses are discussed.

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Chapter 1

Effect of Level of Infusion and Precursor Pool on Estimation of Endogenous N Losses in Pigs using the 15N-Isotope Dilution Method

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Effect of Level of Infusion and Precursor Pool on Estimation of Endogenous N Losses in Pigs using the 15N-Isotope Dilution Method

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Abstract: Two experiments were carried out to study some methodological aspects of the ^{15}N-isotope dilution technique for the determination of endogenous nitrogen (N) losses in pigs. In the first experiment the effect of different ^{15}N-leucine infusion rates (30 mg of ^{15}N-leucine/kg BW/d and 3 mg of ^{15}N-leucine/kg BW/d, respectively) on ^{15}N-enrichment and dilution factors were investigated. In the second experiment the effect of different reference substances (pancreas, small intestine, trichloroacetic acid (TCA)-soluble blood plasma and urine, respectively) to be used as a precursor pool for endogenous N determination were studied in pigs receiving four different diets. A total of 17 ileal cannulated piglets with an average initial weight of 9 kg were used. In Exp. 1 it was found that the ^{15}N-label of total N of the ileal digesta and the TCA-soluble blood plasma were affected by the infusion rate. However, the ratio between the ^{15}N-enrichment of ileal digesta and TCA-soluble blood plasma (dilution factor) expressing the proportion of endogenous N on total N was not influenced. The second experiment revealed similarities in dilution factors calculated from the different precursor pools, except for urine. The choice of a precursor pool remains an important issue for the ^{15}N-isotope dilution method. It seems therefore justified to regard the TCA-soluble fraction of blood plasma as a suitable N-pool for the determination of endogenous N losses in pigs.

Keywords: Piglet; ^{15}N-isotope dilution technique; Dilution factor; Precursor pool

Introduction

The ^{15}N-isotope dilution method is one of the methods used to determine the ileal endogenous N flow in pigs fed N-containing diets (i.e. Souffrant et al., 1981, 1986; De Lange et al., 1990, 1992; Huisman et al., 1992). The use of this method over the last years, however, is subjected of scientific discussions concerning assumptions involved when using this method. According to this method the ratio of ^{15}N-enrichment in ileal digesta to that in a precursor pool (dilution factor) represents the proportion of endogenous N on total N in ileal digesta. The ^{15}N-isotope dilution method assumes that the ^{15}N-enrichment of the precursor pool is similar to that of

endogenous N. Therefore, the selection of the substance whose ^{15}N-enrichment is considered to equal the ^{15}N-enrichment of total endogenous N (Souffrant, 1991) is of particular interest. So far, the trichloroacetic acid (TCA)-soluble fraction of blood plasma has been used as precursor pool in a number of studies (i.e. Hermann et al., 1986; Mosenthin et al., 1991; Jansman, 1993; Makkink, 1993). The validity of this precursor has been questioned by others (De Lange et al., 1992; Moughan et al., 1992). Therefore, the aim of this study is to investigate some methodological aspects of the ^{15}N-isotope dilution method. Aspects studied were: a) time needed to reach a plateau value for ^{15}N-enrichment in the precursor pool, b) effect of level of enrichment of the precursor pool, and c) variation in ^{15}N-enrichment between different precursor pools.

Materials and Methods

Ethics approval for both experiments was given by the TNO-Institute for Nutrition and Food Research and Wageningen Agricultural University Animal Ethics Committees.
The first trial (Exp. 1) was conducted to investigate the effect of time of infusion on ^{15}N-enrichment of TCA-soluble blood plasma and the effect of different ^{15}N-leucine infusion levels on the ^{15}N-enrichment of the TCA-soluble blood plasma and ileal digesta in pigs fed the same diet. In the second trial (Exp. 2) the ^{15}N-enrichment was determined in four different precursor pools (TCA-soluble blood plasma, urine, pancreatic tissue, and small intestinal wall) and in ileal digesta of pigs fed diets based on four different protein sources. The experimental procedures of both experiments were identical. Detailed description of the experimental procedure and composition of the diets of Exp. 2 were presented by Makkink (1993).

Animals, housing and diets. In the experiments, castrated male piglets (Large White) with an age of approximately 6 and 5 wk and an average initial BW of 10 and 8 kg for Exp. 1 and 2, respectively, were individually housed in stainless steel metabolism cages. In total, 17 pigs (6 for Exp. 1 and 11 for Exp. 2), fitted with a post-valve T-caecum (PVTC) cannula (van Leeuwen et al., 1991) and two catheters, one into the external jugular vein (for the infusion) and the other one into the carotid artery (for taking blood samples), were used. The animals were allotted randomly to one of the experimental treatment groups. The ambient temperature was maintained between 23 and 26 °C and the air humidity was kept at 50 to 70%. The ingredient and chemical composition of the experimental diets of both studies are given in Table 1. The total feed allowance was maintained at 480 and 380 g/d for Exp. 1 and 2, respectively. The experimental diets were fed two times a day in similar amounts, at 0800 and

2000 h, respectively. The diets were supplemented with amino acids, minerals, and vitamins according to nutrient requirements of swine (NRC, 1988). Chromic oxide was included in the diets at 1 g/kg as an indigestible marker. Water was available at all times from a low-pressure drinking nipple.

TABLE 1. Ingredient and chemical composition of the diets (%) used in Exp. 1 and 2

	Exp. 1	Exp. 2			
Ingredients	SPC	SMP	SBM	SI	FM
Soya protein conc. (SPC)	34.40	-	-	-	-
Skimmilk powder (SMP)	-	45.50	-	-	-
Soybean meal (SBM)	-	-	34.40	-	-
Soya isolate (SI)	-	-	-	18.20	-
Fish meal (FM)	-	-	-	-	22.15
Cornstarch	39.84	29.60	39.84	52.79	52.91
Dextrose	15.00	15.00	15.00	15.00	15.00
Sunflower/Soya oil	2.00	2.00	2.00	2.00	.50
Cellulose	2.85	5.00	2.85	5.00	5.00
Premix[a]	1.00	1.00	1.00	1.00	1.00
Iodized salt	.50	-	.50	.50	.10
Ground limestone	1.35	.80	1.35	1.40	.75
Monocalcium phosphate	2.10	.50	2.10	2.20	.60
$KHCO_3$	-	.10	-	1.50	1.00
$NaHCO_3$	.40	.30	.40	-	.80
L-lysin HCL	.16	-	.16	.10	-
DL-methionine	.20	.10	.20	.17	.03
L-threonine	.10	-	.10	.04	.05
L-tryptophan	-	-	-	-	.01
Cr_2O_3	.10	.10	.10	.10	.10
Chemical composition, analyzed					
Dry matter	91.36	91.00	89.80	90.70	89.60
CP (N x 6.25)	24.61	15.38	16.52	15.96	16.49
Leucine	1.73	1.57	1.24	1.27	1.18

[a] The premix provided the following per kilogram of feed: 9,000 IU of vitamin A; 1,800 IU of vitamin D_3; 40 mg of vitamin E; 1.36 mg of menadione as dimethyl-pyrimidinol bisulfite; 5 mg of riboflavin; 40 µg of cobalamine; 30 mg of niacin; 12 mg of d-pantothenic acid; 120 mg of choline chloride; 50 mg of ascorbic acid; 2 mg of thiamin; 3 mg of pyridoxine; .1 mg of d-biotin; 1 mg of folic acid; .38 mg of K (KI); .525 mg of Co ($CoSO_4$); .06 mg of Se (Na_2SeO_3); 80 mg of Fe ($FeSO_4$); 254 mg of Cu ($CuSO_4$); 44 mg of Mn (MnO_2); 72.8 mg of Zn ($ZnSO_4$); 40 mg of tylosin.

Experimental design and collection of samples. Both experiments comprised the same consecutive periods as in the studies of Huisman et al. (1992) and Makkink (1993): adaptation to the individual housing in metabolism cages, 5 to 7 d; intestinal cannulation and recovery, 7 to 9 d; catheterization of blood vessels and recovery, 4 to 5 d; infusion of ^{15}N-leucine continuously for 11 d. On d 7, 9, and 11 after the start of the infusion period ileal digesta was collected for 24 h. Ileal digesta were collected directly into a bag fixed to the cannula. Every hour plastic bags were removed. The content was immediately added to the frozen chyme of that animal for that day and stored at -20°C. Blood samples (10 mL) were taken twice a day from the carotid catheter during the infusion period during feeding at 0800 and 2000 h. After sampling the blood was centrifuged (10 min at 1,000 x g, at 4°C). The supernatant was collected and blood plasma protein was precipitated using 20% (wt/vol) TCA (Huisman et al., 1992). The supernatant fluid (TCA-soluble fraction) was stored at -20°C for further analyses (Huisman et al., 1992).

In Exp. 1 the continuous intravenous ^{15}N-leucine infusion was performed at a rate of 30 mg of ^{15}N-leucine (95% ^{15}N-enrichment)/kg BW/d for infusion rate A and 3 mg of ^{15}N-leucine (95% ^{15}N-enrichment)/kg BW/d for infusion rate B with infusion pumps (Perfusor R Dauerinfusionsgerät; Braun Melsungen AG, FRG). In Exp. 2 the continuous i.v. ^{15}N-leucine infusion was performed at a rate of 40 mg of ^{15}N-leucine (95% ^{15}N-enrichment)/kg BW/d. The ^{15}N-leucine was dissolved in a sterile non-pyrogenic physiological saline solution. Approximately 100 mL of this solution was infused daily into each animal.

In Exp. 2 from the start of ^{15}N infusion quantitative urine collections were made daily in containers via funnels underneath the cages. To each container 5 mL of 25% sulphuric acid was added to prevent volatilization of nitrogenous compounds. Twice daily aliquots were taken and frozen at -20°C (Makkink, 1993).

On day 12 three hours after their morning feeding, the animals of Exp. 2 were euthanized. The animals first received inhalation anaesthesia with O_2/N_2O and halothane. The abdomen was opened and pancreas and small intestine were rapidly excised and trimmed of excess fat and lymph nodes, and weighed. Subsequently the samples were frozen and stored at -20°C. Thereafter, the animals were killed.

Chemical analyses. Samples of organs and ileal digesta were freeze-dried, ground through a 1 mm mesh screen and thoroughly mixed prior to analysis. The analysis of DM and N in feed were carried out according to AOAC procedures (1984). The leucine content of the experimental diets was determined using ion exchange chromatography after acid hydrolysis (6 M HCl for 22 h at 100°C).

Before analysis of ^{15}N-enrichment of blood plasma, samples were further pretreated according

to the method described by De Lange et al. (1990). For the determination of the ^{15}N-enrichment in ileal digesta, TCA-soluble plasma, urine, pancreatic tissue, and small intestine the remaining NH_4Cl-solution following titration (Kjeldahl-N analyse) was used. In Exp. 1 this NH_4Cl-solution of TCA-soluble plasma was freeze-dried. For the ^{15}N-enrichment determination 50 ± 10 µg N (Van der Berg, personel communication) of freeze-dried ileal digesta and freeze-dried NH_4Cl-solution of TCA-soluble blood plasma were placed into tin capsules (8 x 5 mm, Fr. Van Loenen Instruments). The tin capsules were combusted in a Total Nitrogen Analyser (Carlo Erba, ANA 1400, Fr. Carlo Erba, Milano, Italy). The Nitrogen Analyser was attached to a mass spectrometer (VG, SIRA-10, VG Isotech Div of Fisons Instr. Middlewich, England) for continuous ^{15}N analyses.

For Exp. 2 the NH_4Cl-solution of ileal digesta, feed, TCA-soluble plasma, urine, pancreatic tissue, and small intestine samples were evaporated, adjusted to a N concentration of 300 to 500 µg/mL (De Lange et al., 1990) and introduced into an emission spectrometer (Isonitromat 5201 or NOI-6, Fa. Statron, Fürstenwalde, Germany) for ^{15}N analysis.

Calculations. The amount of endogenous N can be calculated from the ratio of ^{15}N-enrichment in ileal digesta (E_d) to that in the individually used precursor pool (E_{pl}) using the following formula according to Souffrant et al. (1981) and De Lange et al. (1990):

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

in which N_e is the endogenous N loss; and N_d is the total amount of N in the ileal digesta. The factor (E_d / E_{pl}) is also referred to as the dilution factor. According to Equation 1 the dilution factor represents the proportion of endogenous N on the total N flow passing the terminal ileum. In Exp. 1 TCA-soluble blood plasma represents the precursor pool (E_{pl}). In Exp. 2 this pool is represented by TCA-soluble blood plasma, urine, pancreatic tissue, and the small intestine.

Statistics. The statistical analysis of both experiments were carried out separately. For Exp. 1 the effect of treatment and day of collection on ^{15}N-enrichment and the dilution factor were analyzed by GLM procedure of SAS (1990) according to the following model:

$$Y_{ijkl} = \mu + T_i + A_k(T_i) + D_j + (D_j \times T_i) + e_{ijkl} \qquad [2]$$

where, Y_{ijkl} = dependent variable, μ = overall mean, T_i = treatment (i = 1 and 2), D_j = day of collection (j = 7, 9, and 11), A_k = animal, and e_{ijkl} = residual error. The effect of treatment (T)

was tested against animals within the treatment [A(T)]. Day effect (D) and the effect of interaction of day and treatment (D x T) were tested against the residual error (e). In Exp. 1 treatment represents the ^{15}N infusion rates A and B, respectively.

The comparison of the ^{15}N-enrichment in the total N of the different precursor pools as well as the calculated dilution factors of Exp. 2 were analyzed according to the following model:

$$\mathbf{Y_{ij} = \mu + P_i + e_{ij}} \qquad [3]$$

in which Y_i = dependent variable, μ = overall mean, P_i = precursor pool (i = 1, 2, 3, and 4) and e_i = residual error. Therefore, per animal pooled data of d 7, 9, and 11 of TCA-soluble blood plasma, urine, and ileal digesta as well as pancreatic tissue and small intestine collected on d 12 were used for this comparison. When significant effects of ^{15}N-enrichment and the dilution factor were obtained, differences between the means were compared by the Tukey's test (SAS, 1990).

Results

Pigs remained healthy and consumed their daily allowances throughout the experiment. On the final day of the experiments the mean (± SE) BW of the pigs were 15.8 (± .4) kg and 10.9 (± .1) kg for Exp. 1 and 2, respectively. The dissection after the experimental period showed that the animals had no irregularities due to cannulation and catheterization.

Composition and results on the chemical analyses of the experimental diets of both experiments are given in Table 1. The amount of daily infused ^{15}N-leucine was less than .1% of the daily ingested dietary leucine of the treatment groups of both experiments.

To ensure that the ^{15}N-enrichment of total N in the chosen precursor pool reached a steady state the mean values per infusion rate of Exp. 1 were processed by non-linear regression (Proc NLIN, modified Gauss-Newton method, SAS 1990) according to the formula given by Souffrant et al. (1993). Figure 1 shows the calculated time-course of ^{15}N-enrichment excess of total N in TCA-soluble blood plasma and mean values per treatment group for Exp. 1. Plateau values of .07476 and .01321 ^{15}N-enrichment excess for infusion rates A and B, respectively, were reached after 6 d (144 h) of infusion. Subsequently, ileal endogenous N excretion were estimated from data after d 6 of infusion onwards.

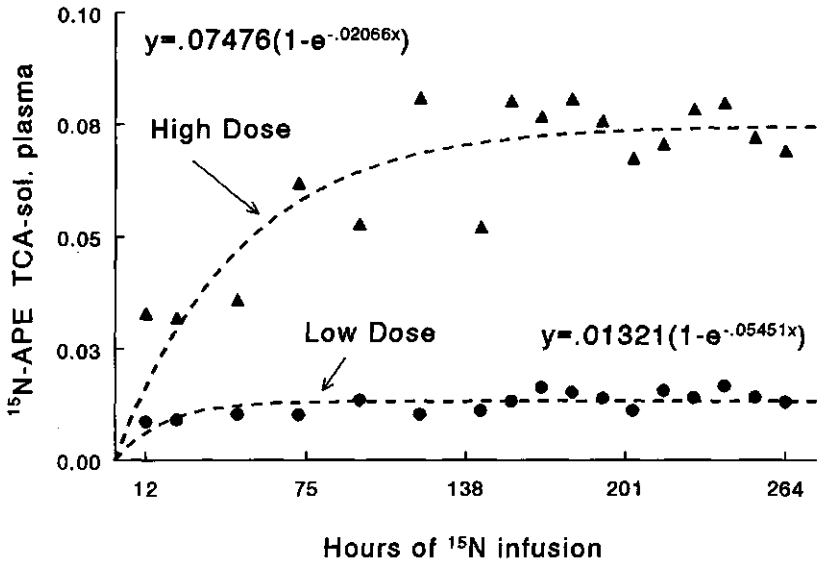

FIGURE 1. Mean observed and calculated time course of ^{15}N-enrichment excess in total N trichloroacetic acid (TCA)-soluble fraction of blood plasma in pigs fed a soybean meal diet and continuously administered ^{15}N-leucine i.v. at different infusion rates: A (High Dosage; ▲) 30 mg/kg of BW/d and B (Low Dosage; ●) 3 mg/kg of BW/d.

Statistical analysis according to the repeated measurement model (Equation 2) of Exp. 1 showed that the ^{15}N-leucine infusion rate (Treatment) influenced significantly ($P < .05$) the total N ^{15}N-enrichment in the TCA-soluble blood plasma and in ileal digesta (Table 2). The dilution factor, however, was not affected ($P > .05$) by the various infusion rates (Table 2). Moreover, there were no collection day effects (Table 2) with regard to the ^{15}N-enrichment of total N in the TCA-soluble blood plasma and ileal digesta as well as the dilution factor ($P > .05$).

TABLE 2. Results of the statistical analyses of the effects of ^{15}N-leucine infusion rates (T), Animal (A), and collection day of blood plasma and ileal digesta (D) and interactions on the ^{15}N-enrichment in TCA-soluble blood plasma and ileal digesta, and the resulting dilution factor of Exp. 1.

Item	T	A(T)	D	D x T	e
^{15}N-enrichment; TCA-soluble blood plasma					
DF	1	4	2	2	8
Mean Squares (x 10^{-3})	15.139	.0667	.0478	.1031	.0432
F probabilities	< .001	.278	.377	.154	
^{15}N-enrichment; ileal digesta					
DF	1	4	2	2	7
Mean Squares (x 10^{-3})	9.275	.0993	.0139	.0073	.0622
F probabilities	< .001	.276	.805	.892	
Dilution factor					
DF	1	4	2	2	7
Mean Squares (x 10^{-3})	.6674	6.678	5.089	17.186	18.989
F probabilities	.857	.835	.772	.447	

In Table 3 the ^{15}N-enrichment excess of the total N in the different precursor pools (TCA-soluble blood plasma, urine, small intestinal tissue, and pancreatic tissue) as well as in ileal digesta of both experiments are presented. Results of statistical analysis of data of Exp. 2 showed that the mean of ^{15}N-enrichment excess of ileal digesta was significantly lower ($P < .05$) than those of TCA-soluble blood plasma, small intestine, and pancreatic tissue for dietary treatments SMP, SBM, and FM of Exp. 2. The data for urine, however, were generally lower and by this closer to those of ileal digesta as compared to the other precursor pools.

For the determination of the endogenous N flow the ratio of the ^{15}N-enrichment value in the ileal digesta to the ^{15}N-enrichment values for the respective precursor pool are important. The mean dilution factors presented in Table 4 are the ratios of the ^{15}N-enrichment from the ileal digesta to various precursor pools in both experiments. With every diet, the dilution factor was highest when the urine was used as reference pool for endogenous secretion. When TCA-soluble plasma was used as reference tissue, dilution factors were not different from dilution factors calculated with the ^{15}N-level of small intestinal or pancreatic tissue.

TABLE 3. The mean ^{15}N-enrichment (%) in total N in the TCA-soluble blood plasma and ileal digesta in pigs fed the experimental diets* and administered continuously ^{15}N-Leucine at different levels (mg/kg BW/d) measured at d 7, 9 and 11 of the infusion period for Exp. 1, and in total N in the TCA-soluble blood plasma, ileal digesta, and urine measured at d 11 and pancreatic tissue and small intestine of d 12 for Exp. 2.

	Experiment 1		SEM	Experiment 2			
Diet	SPC	SPC		SMP	SBM	SI	FM
Infusion level	3	30		40	40	40	40
No. of pigs	3	3		2	3	3	3
Ileal digesta	.0108	.0589	.0028	.13^{c}	.14^{b}	.22^{a}	.15^{b}
TCA-sol. plasma	.0124	.0704	.0022	.25^{a}	.23^{a}	.24^{a}	.23^{a}
Urine	-	-		.18^{b}	.17^{b}	.20^{a}	.13^{b}
Small intestine	-	-		.21^{ab}	.22^{a}	.22^{a}	.24^{a}
Pancreatic tissue	-	-		.21^{ab}	.21^{a}	.23^{a}	.26^{a}
SEM	-	-		.010	.010	.010	.012

* for detailed diet composition see Table 1; SPC means soya protein concentrate, SMP means skimmilk powder, SBM means soybean meal, SI means soya isolate, and FM means fish meal.

^{a,b,c} Values in the same column and in the same row within an experiment followed by different superscripts differ ($P < .05$).

TABLE 4. The ratio of the ^{15}N-enrichment (%) in total N from the TCA-soluble blood plasma and the individual precursor pools given in Table 3 to the mean ileal digesta collected at the distal ileum in pigs fed the experimental diets* and administered continuously ^{15}N-Leucine at different levels (mg/kg BW/d).

	Experiment 1		SEM	Experiment 2			
Diet	SPC	SPC		SMP	SBM	SI	FM
Infusion level	3	30		40	40	40	40
No. of pigs	3	3		2	3	3	3
TCA-sol. plasma	.87	.84	.049	.54^{a}	.59^{a}	.93^{a}	.69^{a}
Urine	-	-		.74^{b}	.83^{b}	1.11^{a}	1.23^{b}
Small intestine	-	-		.64^{ab}	.61^{ab}	.98^{a}	.64^{a}
Pancreatic tissue	-	-		.64^{ab}	.64^{ab}	1.02^{a}	.60^{a}
SEM	-	-		.033	.043	.046	.082

* for detailed diet composition see Table 1; SPC means soya protein concentrate, SMP means skimmilk powder, SBM means soybean meal, SI means soya isolate, and FM means fish meal.

^{a,b} Values in the same column (Exp. 2) followed by different superscripts differ ($P < .05$)

Discussion

Quantification of endogenous protein flows at the terminal ileum is of practical importance for the determination of true ileal protein digestibility of feedstuffs. This is essential to determine which part of ileal digestible protein is related to digestibility and which part is related to endogenous secretion. In addition, this can be essential for the estimation of efficiency of protein gain.

Ileal endogenous protein. Considerable amounts of endogenous nitrogenous compounds enter the mammalian gut (Souffrant, 1991). It comprises N from various sources, such as enzymes, mucoproteins, desquamated epithelial cells, serum albumins, peptides, amino acids, amines, and urea (Moughan et al, 1992). From literature data on separate sources of endogenous N secretion Auclair (1986) estimated that the proportion of the entire endogenous N secretion in pigs consists of approximately 10% from salivary and gastric secretion, 17% from pancreatic secretion, 9% from bilary secretion, 57% from small intestinal secretion and 7% from sloughed cells. During the passage through the digestive tract these compounds are subjected to digestion and absorption (Buraczewski, 1980). The amount and composition of endogenous nitrogenous compounds at the terminal ileum is thus the net result of processes occurring in the upper digestive tract. The reabsorption rate for nitrogen secreted endogenously up to the terminal ileum was found to range from 70% to 79% (Low, 1982; Souffrant et al., 1986; Krawielitzki et al., 1990; Souffrant et al., 1993). The mucoproteins are a major source of endogenous protein at the terminal ileum of pigs according to Moughan and Schuttert (1991), Butts et al. (1993), and Lien et al. (1993).

The ^{15}N-dilution method. The principle of the stable isotope (^{15}N) dilution method as used in the present study to differentiate between endogenous and non-digested dietary protein in the digested tract is based on the isotopic ratio in digesta and precursor pool. To measure the ileal endogenous protein mixture a tracer is required which labels all endogenous nitrogenous compounds. Principally this can be done by using stable isotopes of carbon, hydrogen and nitrogen. However, carbon- and hydrogen tracer may leave the metabolic protein pool by expired CO_2 or water, respectively. Furthermore, carbon and hydrogen are not only associated with protein as nitrogen is, but also with other metabolites.

The used ^{15}N-isotope dilution method in the present study, involved a continuous infusion of stable N, is based on the concept that any ^{15}N-enrichment in the digesta must have been derived from the animal itself. As used in the present study, there are several advances using the tracer ^{15}N-leucine to label the animals metabolic N pool. Leucine is a stable amino

acid and has no major metabolic role other than protein synthesis (Moughan et al., 1992). Metabolic processes, especially transamination, result in the ^{15}N incorporation into plasma amino acids and other N-containing compounds. The ^{15}N-enrichment is highest in that amino acid that is used to administer the ^{15}N isotope (Matthews et al., 1978, 1981; De Lange et al., 1992). However, amino acids, that do not participate significantly in transamination, as lysine and threonine, show little or no incorporation of ^{15}N (Matthews et al., 1978, 1981, Schadereit et al., 1986). Gebhardt et al. (1983) recommended a mixture of various ^{15}N-labelled amino acids to overcome these problems. This, however, is hardly feasible for a continuous 10 d infusion in pigs. According to Souffrant et al. (1981) ^{15}N-labeled leucine administered via continuous i.v. infusion can successfully be used to label the metabolic N pool of the pig.

Steady-State. The establishment of steady-state conditions is a basic requirement of the ^{15}N-isotope dilution technique. This can be achieved by continuous infusion of ^{15}N-leucine as used in the present experiment. Reaching steady-state conditions in the case of the ^{15}N-dilution method can be expressed in two different ways:

1) A steady-state is achieved when the level of ^{15}N-enrichment in the N-pool does not change substantially after a certain time. In the present study the estimated ^{15}N-enrichment of the total N-pool of TCA-soluble blood plasma and ileal digesta (Exp. 1) remained fairly constant during d 7, 9, and 11 of the infusion period ($P = .377$ and $P = .805$ for TCA-soluble blood plasma and ileal digesta, respectively; Table 2). Calculation of the plateau value of the ^{15}N-enrichment in the TCA-soluble blood plasma (Figure 1) gave the steady state value independently on the used infusion rates after 6 days of infusion. Results from literature, using a continuous infusion of ^{15}N-leucine at a daily rate of 40 mg/kg BW weight via one of the jugular veins (Souffrant et al., 1981, 1986; de Lange et al., 1990, 1992; Mosenthin et al., 1991; Souffrant, 1991; Huisman et al., 1992) supported these findings.

2) Under steady state conditions, the ratio of the degree of ^{15}N-enrichment excess of N in chyme to that of the precursor pool should not change. In the present study (Exp. 1) it was found that the ratio of ^{15}N-enrichment of total N in ileal digesta to that in TCA-soluble blood plasma showed similarity ($P > .05$; Table 2) over time of infusion (d 7 to 11) of labelled leucine. This indicates that any changes in ^{15}N-enrichment with time of infusion (d 7 to 11) occurred in concert between the TCA-soluble blood plasma and digesta total N.
Consequently, the relative contribution of endogenous N to total N in ileal digesta does not appear to differ greatly over the infusion period as long as a direct relation to the chosen precursor N-pool consists. Moreover, Exp. 1 of the present study demonstrated that the used infusion rates did not affect the relative contribution of endogenous N to total N in ileal digesta when one diet was administered.

Precursor Pool. The isotope dilution method assumes that the labelling of endogenous

N is similar to that of the precursor N-pool. This means that the ^{15}N-enrichment of the precursor is at a similar level then that of the endogenous protein (N x 6.25) mixture excreted in the digestive tract. Therefore, it is assumed that the ^{15}N-enrichment in a precursor pool is similar to ileal endogenous protein synthesized and secreted and also to ileal endogenous nitrogenous compounds which are found at the terminal ileum. In isotope studies the metabolic N-pool is often referred to as the precursor pool for protein synthesis.

In the present study, the ratios of specific activity in ileal digesta and in the potential precursor pools (dilution factors; Table 4) were similar for TCA-soluble blood plasma, small intestinal and pancreatic tissue within each dietary treatment. These pools had similar degrees of labelling, reflecting apparent similarity in the degree of incorporation of ^{15}N-label in the nitrogenous compounds. For urine, however, different dilution factors were found (Table 4; Exp. 2). This is therefore considered unsuitable for calculating endogenous proportion in the total ileal N flow.

The present experimental design allowed examination of the relative suitability of the potential precursor pools samples. By definition, the ^{15}N-label in total N of ileal digesta can never be higher than that in its metabolic or precursor pool, because true digestibility is always higher than apparent digestibility. Consequently, the estimated dilution factor can have a maximal range from 0 to 1. Furthermore it can be assumed that the ileal endogenous flow of N for the various dietary protein treatment groups consists of a basal and a variable endogenous N flow. The basal rate is associated with dry matter intake and the variable rate is associated with specific endogenous stimulating compounds like antinutritive factors (ANF). Differences in the experimental diets with regard to additional endogenous N losses are mainly considered related to the protein source used. Variable endogenous N flow means a specific level due to protein quality or included ANF's in the protein source. Other aspects in composition are much more similar between the diets. The basal amount of daily ileal endogenous N loss can be calculated according to the equation given by Butts et al. (1993):

endogenous ileal N excretion (mg/d) = 2.8 x food dry matter intake (g/d) + 122

With a mean dry matter intake of 439 and 343 g/d a basal ileal endogenous N excretion of 1.35 and 1.08 g/d was calculated for Exp. 1 and 2, respectively. The mean daily total N for both treatment groups of Exp. 1 was 2.62 g/d (Schulze, unpublished results). Therefore, the dilution factor, the ratio of endogenous and total N flow (1.35/2.62), should be in a range from .52 to 1. This confirm the found results for Exp. 1 (Table 4). Similarly the total N flow of dietary treatments used in Exp. 2 were 1.46, 2.36, 2.10, and 2.71 g/d (Makkink, 1993) and the dilution factors will range from .74, .46, .51 or .40 to 1 for the dietary treatments SMP, SBM, SI and FM, respectively. Differences comparing these results with findings from the present study can be related to effects of the included protein source in the dietary treatments of Exp.

2. The use of the urinary N-pool, however, delivered unsatisfactory values independent of dietary treatments. The ^{15}N-labelling of urine depends largely on the protein utilization and therefore on protein intake. The N in urine depends on catabolized body protein and catabolized food protein. This means that urinary ^{15}N-enrichment does not need to be similar to that of the endogenous pool. Consequently urine can not be used as a precursor pool to estimate endogenous N losses (Herrmann et al., 1986).

In the present study (Exp. 2) the ^{15}N-label of total N in the TCA-soluble blood plasma corresponds well with those of pancreatic tissue and small intestinal wall within the dietary treatments (Table 3). According to Krawielitzki et al. (1990) the ^{15}N in the TCA-precipitate of the pancreas can also be used as a suitable indicator for the ^{15}N level of endogenous protein. During the development of the ^{15}N-isotope dilution method it was found that the TCA-soluble fraction of blood plasma can be used as precursor N-pool for endogenous N excretion (Souffrant et al., 1981, 1993), because of its resemblance to the total body N-pool with respect to enrichment. The TCA-supernatant of the blood plasma consists of free amino acids, urea and other NPN (non protein nitrogen)-compounds. Thus, urea also contributes to the ^{15}N-enrichment of the total N-pool. It has been suggested that because of this an underestimation of the endogenous protein (N x 6.25) flow is obtained (De Lange et al., 1992). The urea flow from the blood into the digestive tract, however, can be as high as 32% (Thacker et al., 1984) and 50% (Rerat and Buraczewska, 1986; Bergner et al., 1986). According to Mosenthin et al. (1992a) stomach and small intestine represent the main sites of urea secretion in the gastrointestinal (GI) tract of pigs. Urea and ammonia constitute only about .57% of the total N in the ileal digesta under protein-free alimentation (Moughan and Schuttert, 1991). This level depends on the microbial activity. It was shown that endogenous urea was used by intestinal bacteria and (partly) incorporated in synthesized microbial amino acids (Deguchi et al., 1978a, 1978b, 1980; Mosenthin et al., 1992b). This means, in agreement with Rerat et al. (1979), that urea can contribute to endogenous protein present in the GI tract.

On theoretical grounds the direct precursor pool for endogenous protein (N x 6.25) secretion is the sum of the intracellular free, tRNA bound or secretory protein bound N-pools in gut epithelial, pancreatic acinar tissue and from the other tissues involved in the endogenous protein secretion. Measurement of the amino acid tRNA enrichment, however, is difficult and laborious. Sampling the appropriate tissue free amino acid pool has also been found to be an acceptable alternative (Assimon and Stein, 1992).

From the findings of the present study it can be concluded that in the present form (i.v. infusion of ^{15}N-leucine and estimation the ^{15}N-enrichment in the total N-pool of TCA-soluble blood plasma and ileal digesta) the isotope dilution method is suitable to determine the endogenous N excretion passing the terminal ileum in pigs. Comparable investigations of the

ileal endogenous N flow measured with the ^{15}N-isotope dilution and the homoarginine method (Hagemeister and Roos, 1991) or the peptide alimentation method (Schulze et al., 1994) support this conclusion. In addition, it can be concluded that a reduction of the infusion rate of ^{15}N-leucine to about 3 mg/kg BW/d do not have any effect on the calculation of the endogenous N flow.

Implications

Future investigations onto estimation of endogenous N secretion with isotopes need correct precursor pools such as the intracellular fluid from secretory cells along the digestive tract. Ideally, secretory cells from representative parts of all the secretory tissues involved in endogenous secretion need to be labelled. Also, the sloughing of non-secretory gut wall cells should be taken into account as well as the contribution of the small intestinal microflora. The present study shows that the TCA-soluble fraction of the plasma is an alternative to measure ^{15}N-enrichment of endogenous N secretions.

Comparison of the ^{15}N-isotope dilution method using the ^{15}N enrichment in the total N TCA-soluble fraction of blood plasma with other methods also shows that this is an acceptable precursor pool. The ^{15}N-isotope dilution method may be used therefore to study factors that affect the ileal endogenous protein recovery in pigs fed protein containing diets.

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Chapter 2

The ^{15}N-Isotope Dilution Method for Determining Ileal Endogenous Nitrogen Excretion in the Young (10 kg Liveweight) Pig

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Abstract: A study was conducted with four crossbred 5-wk old castrated male pigs at an average BW of 10 kg to determine the endogenous amino acid and nitrogen flows at the terminal ileum using the peptide alimentation and ^{15}N-isotope dilution methods. The pigs were fitted with a post-valvular T caecal cannula and two indwelling blood catheters. They were fed a corn starch-based semisynthetic diet formulated to contain enzymically hydrolyzed casein (EHC) as the sole source of protein at twice their maintenance requirement for energy. Digesta from the EHC-fed animals were centrifuged and ultrafiltrated after collection and the precipitate plus retentate fraction (MW > 10,000 Da) was used to determine the endogenous amino acid and nitrogen flows. To estimate the endogenous N flow at the terminal ileum of these pigs using the ^{15}N-isotope dilution method a constant 10 day ^{15}N-leucine infusion was performed at a daily rate of 5.04 mg of ^{15}N-leucine (95% ^{15}N enrichment) kg^{-1} bodyweight. The mean ^{15}N-enrichment above background for the TCA-soluble blood plasma and ileal digesta nitrogen pools were 0.0249 and 0.0178, respectively. There were no statistically significant differences of ^{15}N-enrichment excess between the days of ileal collection. The results demonstrated that glutamic acid, aspartic acid, proline and serine formed about 50% of the total ileal endogenous amino acid flow. The total daily amount of endogenous amino acid flow at the terminal ileum was 6.2 g/d. The endogenous ileal N flow determined with the peptide alimentation and the ^{15}N-isotope dilution methods were similar (P = .40) with 1.37 and 1.17 g/d, respectively. The proportion of endogenous N on the total N flow passing the terminal ileum was 83% and 72% for the peptide alimentation and the ^{15}N-isotope dilution method, respectively.

Key words: pig, endogenous ileal loss, amino acid, nitrogen, peptide alimentation, ^{15}N-isotope dilution

INTRODUCTION

Quantification of endogenous amino acid losses at the terminal ileum of the pig is of practical importance for the determination of amino acid requirements and also for calculation of the true ileal amino acid digestibilities of feedstuffs. The endogenous loss of nitrogen (N) and amino acids (AA) from the gastrointestinal tract of animals has traditionally been determined after feeding them a protein-free diet. Recent studies (Moughan and Rutherfurd 1990; Darragh *et al* 1990; de Lange *et al* 1990; Butts *et al* 1991, 1993a), however, have shown that the endogenous loss of amino acids from the small intestine of the simple-stomached animal is higher following the feeding of diets containing protein or peptides.

A recently developed method for determining endogenous amino acid flow at the terminal ileum, whereby the influence of dietary peptides is present, is the peptide alimentation ultrafiltration method (Moughan *et al* 1990, Butts *et al* 1991). In determining endogenous losses under peptide alimentation, the animal is fed a semi-synthetic diet containing enzymically hydrolyzed casein (EHC) as the sole nitrogen source. Ileal digesta are collected from the animal, and the endogenous protein (MW > 10,000 Da) is separated by centrifugation and ultra-filtration. The precipitate plus the high-molecular -weight fraction (MW > 10,000 Da) resulting from ultrafiltration of the supernatant, provides a measure of endogenous amino acid flow. If dietary amino acids and small peptides are not absorbed in the gut, they are discarded in the low-molecular-weight (MW < 10,000 Da) fraction. The latter approach is direct and does not rely on isotopes as markers. Moreover, it has been shown to give estimates of endogenous amino acid flow similar to those determined directly after the ingestion of whole protein (Moughan and Rutherfurd 1990; Butts *et al* 1993a). The EHC peptide alimentation method was accepted, in the present study, as a suitable baseline method for determining endogenous amino acid losses in the pig fed a casein containing diet.

An alternative but more general, indirect approach to determining endogenous losses in the pig is the isotope dilution method (Souffrant *et al* 1981). In the ^{15}N-isotope dilution method, the contribution of endogenous nitrogen to total nitrogen in the ileal digesta is bound on the ratio of ^{15}N-enrichment in ileal digesta and in an appropriate precursor N-pool, assuming that the ^{15}N-enrichment of endogenous ileal nitrogen is similar to that in the precursor pool. Central to this method, however, is the choice of a suitable precursor pool and there is doubt to which, of the alternative pools is suitable (Moughan *et al* 1992).

The aim of the present study was to determine endogenous nitrogen excretion at the terminal ileum of the young pig receiving a casein based diet, using the ^{15}N-isotope dilution method, and to compare the estimate obtained with that from the EHC peptide alimentation ultrafiltration method. A secondary objective was to obtain estimates of ileal endogenous amino

acid flow from the young (10 kg liveweight) pig.

EXPERIMENTAL

Ethics approval for this study was given by the Wageningen Agricultural University and TNO-Institute for Nutrition and Research Animal Ethics Committees.

Four 5-week-old crossbred (Dutch Landrace x Yorkshire) castrated male pigs were obtained from a commercial breeding farm. The animals were individually housed in smooth-walled metabolism cages (1.2 m x 1.2 m) in a temperature controlled room (23 to 26°C; 50 to 70% relative humidity). Following an 8 to 9 day adaptation period, the pigs underwent surgery for the implantation of a post-valve T-caecum (PVTC) cannula, according to the procedure of van Leeuwen *et al* (1991). During recovery from surgery, the pigs were fed to appetite a semi-synthetic casein based diet containing 29 g N/kg. Ten days following surgery two indwelling catheters were surgically implanted, one into the external jugular vein (for taking blood samples) and the other one into the carotid artery (for the infusion of ^{15}N-Leucine solution), according to the method of Weirich *et al* (1970). The animals were allowed 24 h to recover from the second surgical manipulation before starting a ten-day experimental period. At the start of this period the mean (± SE) liveweight was 9 (± 0.5) kg. On the final day of the experiment the mean liveweight of the pigs was 11 (± 0.6) kg.

Throughout the 10 day experimental period, the pigs were given a semi-synthetic enzymically hydrolysed casein (EHC)-based diet (Table 1) twice daily (08.00 and 20.00) in equal portions. The level of food intake (approximatly 375 g/d) given to the animals during the experiment provided around two times their maintenance requirement for energy (Agricultural Research Council 1981). Amino acids, vitamins, and minerals were provided in quantities that met the growing pig's requirements (NRC 1988). Chromic oxide (1 g/kg) was included in the diet as an indigestible marker. The diet was mixed with water (1:1, w/v) immediately prior to feeding and fresh water was available for 30 min after each meal.

One day after insertion of the catheters a constant 10 day ^{15}N-leucine infusion was performed at a daily rate of 5.04 mg of ^{15}N-leucine (95% ^{15}N enrichment) kg^{-1} bodyweight. To minimize the time taken to reach the steady state of ^{15}N enrichment in the precursor pool the animals were primed with 0.21 mg of ^{15}N-leucine kg^{-1} bodyweight. The ^{15}N-leucine was dissolved in a sterile non-pyrogenic physiological saline solution. About 50 ml of this solution was infused daily in each animal. Accurate perfusion pumps (Fr. B. Braun Melsungen AG, FRG) were used to infuse the solution. To infuse a free moving animal continuously the 'Swivel-technique' as described by Van Kleef (1993) was used.

TABLE 1. Ingredient composition (g/kg air dry weight) of the experimental diet

Ingredient	Composition
Enzymically hydrolysed casein[1]	180
Maize starch	521
Soya bean oil	25
Glucose	148
Purified cellulose[2]	50
Premix[3]	10
Minerals[4]	61
DL-methionine	1
Cystine	2
L-threonine	1
Chromic oxide	1

[1] Sigma Chemical Company, St Louis, MO, USA. Type I from bovine milk. Molecular weight < 5,000 Da.
[2] Arbocell B 800, Fr. Jrettenmayer & Söhne (Germany).
[3] The vitamin/mineral mix provided the following per kilogram of feed: 9,000 IU of vitamin A; 1,800 IU of vitamin D_3; 40 mg of vitamin E; 1.36 mg of menadione as dimethyl-pyrimidinol bisulfite; 5 mg of riboflavin; 40 µg of cobalamine; 30 mg of niacin; 15 mg of d-pantothenic acid; 120 mg of choline chloride; 50 mg of ascorbic acid; 2 mg of thiamin; 3 mg of pyridoxine; .1 mg of d-biotin; 1 mg of folic acid; .38 mg of K (KI); .525 mg of Co ($CoSO_4$); .06 mg of Se (Na_2SeO_3); 80 mg of Fe ($FeSO_4$); 254 mg of Cu ($CuSO_4$); 44 mg of Mn (MnO_2); 72.8 mg of Zn ($ZnSO_4$); 40 mg of tylosin.
[4] Contributed the following per kg of feed: $CaCO_3$, 14.5 g, Monocalciumphosphate, 21 g; NaCl, 5 g; $KHCO_3$, 16.5 g; $NaHCO_3$, 2 g; MgO, 2 g.

Ileal digesta were collected continuously for 12 hours on days 8, 9, and 10 of the infusion period. The digesta were collected in small plastic bags attached to the cannula. The bags were emptied hourly and the digesta immediately frozen and stored at -20°C. Prior to chemical analysis, the digesta for each animal were freeze-dried and finely (< 1 mm) ground.

For the determination of endogenous ileal amino acid (AA) flow by the peptide alimentation ultrafiltration method, 3 g of freeze-dried ileal digesta were rehydrated in 20 ml deionised water. The samples were left for 1 h at 5°C. The digesta were than centrifuged for 20 min at 1600 x g and then subjected to ultrafiltration according to the method described by Butts *et al* (1991). The high molecular weight fraction (mol wt > 10,000 Da, the retentate) was added to the precipitate, and subsequently freeze-dried. The low molecular weight fraction was discarded.

Prior to the start of the 10 day infusion period, one blood sample (10 ml) was taken from each animal to determine the background ^{15}N-enrichment of the trichloroacetic acid (TCA)-soluble blood plasma. On days 8, 9 and 10 of the infusion period blood samples (5 ml)

were taken three times a day, at 09.00, 15.00 and 21.00, respectively. Immediately after sampling, the blood was centrifuged (10 min 1,000 x g). The plasma from each animal was pooled per day and stored at -20°C. The precipitate was discarded. Before chemical analysis for ^{15}N-enrichment, duplicate samples were taken from each sample. Forty percent (w/v) TCA (0.1 ml) was added to 0.5 ml of the pooled plasma samples and mixed. After overnight (16 h) storage at 4°C, this mixture was centrifuged at 3000 x g for 20 min at 4°C. The precipitate was discarded, and the pH of the supernatant was adjusted to pH 7. Subsequently this mixture was freeze-dried and sampled for analyses.

Chemical Analysis

Total nitrogen (N) and dry matter (DM) were determined in the diet, freeze-dried ileal digesta and processed ileal digesta precipitate plus retentate samples following AOAC (1984) procedures. Chromium was determined in the diet and in the ileal digesta by the method of Bosch *et al* (1989). Amino acids were determined in the processed ileal digesta precipitate plus retentate samples, after hydrolysis using a Pharmacia LKB Alpha Plus amino acid analyser. Samples of 5 to 7 mg of freeze-dry matter were hydrolysed in 500 µl of 6 M HCl plus 1% phenol for 24 h at 110 ± 2°C in glass tubes sealed under vacuum. Cystine and methionine were determined following oxidation with performic acid prior to acid hydrolysis (Moore 1963). Free amino acid molecular weights were used to convert the amino acid concentrations from nmoles to mg. All analyses were performed in duplicate.

The ^{15}N-enrichments of total N in ileal digesta, diet and TCA-soluble plasma were measured using a dual inlet isotope ratio mass spectrometer (VG Isotech, Fison Instruments, Middlewhich, U.K.). Fifty ± 10 µg N of freeze-dried ileal digesta and TCA-soluble blood plasma and of the diet were placed into tin capsules (8 x 5 mm, Fr. Van Loenen Instruments). The tin capsules were combusted in a Total Nitrogen Analyser (Carlo Erba, ANA 1400, Fr. Carlo Erba, Milano, Italy) which was attached to the mass spectrometer. Samples were determined in duplicate, resulting in a precision of ± 0.001 ^{15}N atom percent excess (APE).

Data Analysis

Total N and dry matter flows at the terminal ileum were determined on triplicate for each pig, using the 12 h pooled digesta samples, collected on days 8, 9 and 10 of the experimental period, according to the chromium ratio marker method as described by Furuya and Kaji (1992). The daily endogenous AA and N flows at the terminal ileum, determined using the peptide alimentation method, were calculated using the following equation:

Endogenous AA/N flow = **AA/N content of processed digesta x ileal DM flow** [1]
(g/d) (g/kg DM) (kg/d)

and for the endogenous AA and N flows relative to the ingestion of 1 kg of food dry matter (DMI)

Endogenous AA/N flow = **Endogenous AA/N flow x Food dry matter intake^{-1}** [2]
(g/kg DMI) (g/d) (kg/d)

For the determination of endogenous loss using the ^{15}N-isotope dilution method, endogenous ileal N flows were calculated using the following equation:

$$N_e = N_d \times [(E_d - E_{nf})/(E_{pl} - E_{npl})] \quad [3]$$

where N_e is the endogenous N loss (g/d and g/kg DMI); N_d is the total amount of N in the ileal digesta (g/d and g/kg DMI); E_d is the ^{15}N-enrichment in ileal digesta; E_{nf} is the background ^{15}N-enrichment in the diet; E_{pl} is the ^{15}N-enrichment in the TCA-soluble blood plasma; and E_{npl} is the background ^{15}N-enrichment in the TCA-soluble blood plasma. The factor $[(E_d - E_{nf})/(E_{pl} - E_{npl})]$, referred to as the dilution factor, was calculated for each animal for each individual day of ileal digesta collection.

Daily values for the ^{15}N-enrichment excess were subjected to non-linear regression (Proc NLIN, modified Gauss-Newton method, SAS 1990) according to the formula given by Souffrant *et al* (1993), to demonstrate that the ^{15}N-enrichment of total N in the chosen precursor pool had reached a steady state.

Statistical Analysis

The ^{15}N-enrichment data for total N in the TCA-soluble blood plasma and in ileal digesta and the dilution factors were subjected to an one-way analysis of variance.
The effect of method on endogenous N flow at the terminal ileum was examined by the GLM procedure of SAS (1990) according to the following model:

$$Y_{ijkl} = \mu + M_i + A_k + D_j + (D_j \times M_i) + e_{ijkl} \quad [4]$$

where, Y_{ijkl} = dependent variable, μ = overall mean, M_i = method (i= 1, the ^{15}N-isotope dilution method; 2, EHC method), D_j = day of collection (j= 8, 9, and 10), A_k = animal (k= 1, 2, 3, 4),

and e_{ijkl} = residual error. "A" represents the animal effect. The effect of method (M) was tested against the between animals variation (A). Day effect (D) and the effect of interaction of day and method (D x M) were tested against the residual error (e). The significance of the method on the parameter estimates was determined using the F-value which was calculated for the effect of method tested against animals. This method is similar to that reported by Schrama *et al* (1993) and is a powerful test to destinguish between method and time. Animals and residuals were assumed to be normally distributed.

RESULTS

The pigs remained healthy, readily consumed the experimental diet and increased in body weight. The mean ileal dry matter and total N excretions (mg/d and mg/kg DMI), determined with reference to the chromium marker, are presented in Table 2.

TABLE 2. Mean dry matter (DM) and total nitrogen (N) flows at the terminal ileum of the growing pig fed an enzymically hydrolysed casein based diet.

	g/d	SE[2]	g/kg of DMI	SE[2]
Ileal DM flow	53.9	3.21	143.9	7.94
Total ileal N flow	1.64	0.106	4.37	0.239

[2] Standart error of the mean; n = 4.

The mean endogenous AA flows (mg/d and mg/kg DMI) at the terminal ileum for growing pigs, determined using the peptide alimentation ultrafiltration method, are presented in Table 3. In order of decreasing abundance, the greatest quantities of endogenous AA found in ileal digesta were glutamic acid (Glu), aspartic acid (Asp), proline (Pro) and serine (Ser). These dispensable AA formed more than 78% of the total amount of dispensable AA and about 50% of the total amount of endogenous AA measured at the terminal ileum. The proportions of indispensable and dispensable AA on the total amount of estimated endogenous AA flow at the terminal ileum are about 37% and 63%, respectively.

TABLE 3. Mean endogenous amino acid flows[1] at the terminal ileum of the growing pig determined using the peptide alimentation, ultrafiltration method.

	Endogenous Flow of Amino Aids			
	mg/d	SE[2]	mg/kg of DMI	SE[2]
Indispensable Amino Acids				
Arginine	179	12.9	481	40.3
Histidine	161	19.4	436	61.1
Isoleucine	236	28.6	638	87.0
Leucine	329	23.0	883	78.2
Lysine	246	32.7	663	94.4
Methionine	127	15.1	342	44.0
Cystine	163	30.4	432	77.5
Phenylalanine	189	12.5	509	45.2
Tyrosine	127	7.3	341	26.8
Threonine	413	30.4	1,108	98.0
Valine	385	33.5	1,030	97.4
Dispensable Amino Acids				
Alanine	249	25.5	671	76.1
Aspartic acid	699	54.8	1,878	172.4
Glutamic acid	1,344	128.8	3,610	389.4
Glycine	284	13.1	762	44.6
Proline	607	72.9	1,630	215.0
Serine	478	48.3	1,287	150.9
Total	6,216	522.5	16,701	1,623

[1] The pigs were given an enzymically hydrolyzed casein based diet and the digesta were centrifuged and ultrafiltered prior to chemical analysis. [2] Standart error of the mean; n = 4.

In the present study the background ^{15}N enrichment of the N in the TCA-soluble blood plasma and the ingested feed were 0.3680 and 0.3690, respectively. The mean estimated ^{15}N-enrichment excesses for the TCA-soluble blood plasma and ileal digesta nitrogen pools were 0.0249 and 0.0178, respectively. As shown in Table 4, the plasma ^{15}N-enrichment excess remained relatively constant over the days of ileal collection (days 8, 9, and 10 of the infusion period) and there were no statistically significant differences between days.

TABLE 4. Mean ^{15}N-enrichment excess[1] in the plasma-free and in the terminal ileal digesta nitrogen of growing pigs given a continuous 10-day infusion of ^{15}N-labelled leucine and receiving an enzyme-hydrolysed casein based diet.

	^{15}N-enrichment excess		
Day of Collection	Blood Plasma	Ileal Digesta	Dilution Factor[2]
8	0.0246[a]	0.0165[a]	0.67[a]
9	0.0246[a]	0.0187[b]	0.76[b]
10	0.0255[a]	0.0182[b]	0.71[ab]
SE	0.0004	0.0004	0.03

[1] The background ^{15}N enrichment in total N of TCA-soluble blood plasma and of feed were 0.3680 and 0.3690, respectively.
[2] Dilution factor = ^{15}N-enrichment excess in digesta/^{15}N-enrichment excess in plasma free N-pool.
[a,b] Means (n=4) with different superscript are significantly different ($P < 0.05$).

The time-course for mean ^{15}N-enrichment excess of total N in the TCA-soluble blood plasma is presented in Figure 1. Although the calculated plateau values for TCA-soluble blood plasma ^{15}N-enrichment excess of .0274 was not reached the estimated daily means were close to the curve and the plateau value was approximated.

Under steady state conditions, the ratio of ^{15}N-enrichment of the digesta total N to the plasma ^{15}N-enrichment should be constant. In the present study it was found that the ratio of ^{15}N-enrichment of total N in ileal digesta to that in TCA-soluble blood plasma (dilution factor) was similar over time of infusion (d 8 to 10) of labelled leucine. The overall mean dilution factor (± SE) for the digesta collection days 8, 9 and 10 was 0.72 (± 0.02). The daily dilution factors were not significantly different from the mean dilution factor (Table 4). This indicates that any changes in ^{15}N-enrichment with time of infusion (d 8 to 10) occured in concert between the TCA-soluble blood plasma and digesta total N. Consequently, the relative contribution of endogenous N to total N in ileal digesta did not appear to differ greatly over the infusion period. Statistical analysis according to the repeated measures model (Equation 4) showed that there was no significant effect of time ($P > 0.05$) on endogenous N flow at the terminal ileum, estimated using either the peptide alimentation or the ^{15}N-isotope dilution method (Table 5a). The 7 day adaptation period to the experimental feed and the continuous ^{15}N-infusion was adequate for adaptation of the animals.

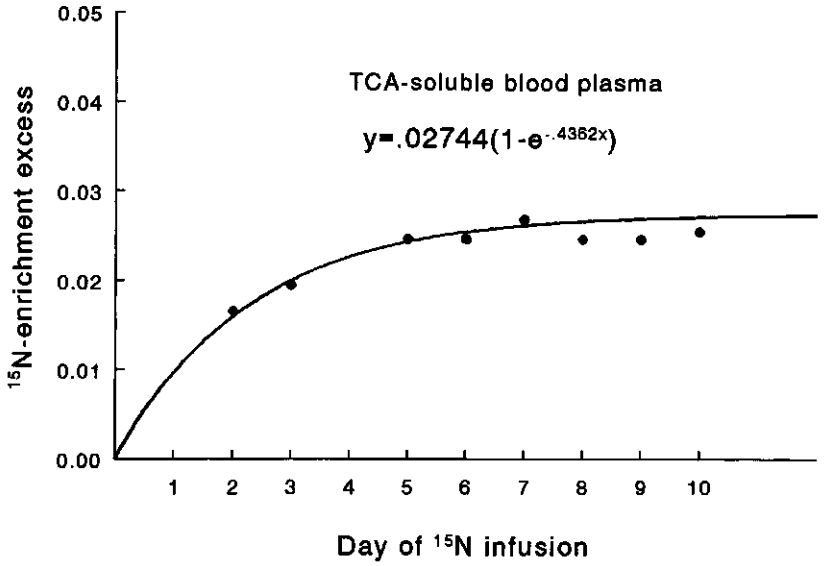

FIGURE 1. Exponential time course for the ^{15}N-enrichment excess of the TCA-soluble total N fraction of blood plasma in pigs fed an enzymically hydrolysed casein based diet and continuously administered ^{15}N-leucine (5.04 mg kg^{-1} bodyweight day^{-1}), (▲ = means of observations).

TABLE 5a. Results of the statistical analyse for the effect of method (M), Animal (A), and collection day of ileal digesta (D) and their interactions on endogenous N flow at the terminal ileum of the pig.

Item	M	A	D	D x M	e
Endogenous N flow; g/d					
Degrees of Freedom	1	3	2	2	10
Mean Square	.229	.240	.023	.004	.070
F probability	.400	.060	.723	.946	
Endogenous N flow; g/kg DMI					
Degree of Freedom	1	3	2	2	10
Mean Square	1.459	1.185	.204	.029	.478
F probability	.348	.121	.664	.942	

The data on mean endogenous N flows (g/day and g/kg DMI) determined with the peptide alimentation and with ^{15}N-isotope dilution methods are presented in Table 5b. The amounts of endogenous ileal N flow for the two methods, were not statistically significantly different ($P < 0.05$). Endogenous N as a proportion of the total N flow at the terminal ileum as estimated with the peptide alimentation or the ^{15}N-isotope dilution methods, were 83 and 71%, respectively.

TABLE 5b. Mean endogenous N flows at the terminal ileum of the growing pig determined using the ^{15}N-isotope dilution and peptide alimentation method.

	Endogenous Nitrogen Flows			
Method	g/d	SE	g/kg of DMI	SE
Peptide alimentation	1.37	.184	3.64	.434
^{15}N-isotope dilution	1.17	.079	3.12	.221

DISCUSSION

The endogenous flow of protein (N x 6.25) and amino acids from the gastrointestinal tract of animals has traditionally been determined after feeding the animal a protein-free diet. This approach may be questionned, because it creates a physiologically abnormal state (Low 1980), which may lead to a changed rate of whole body protein synthesis (Millward *et al* 1976; Muramatsu 1990). Moreover, the protein-free nutritional status may affect the amount of protein entering the gut (De Lange *et al* 1990; Chung and Baker 1992; Moughan *et al* 1992; Butts *et al* 1992, 1993a).

Therefore, a genuine more acceptable method for routinely determining endogenous ileal protein flow under conditions of protein feeding is needed. The ^{15}N-isotope dilution method, one of the most common methods used to estimate endogenous nitrogen flows (i.e. De Lange *et al* 1990; Souffrant *et al* 1991, 1993; Krawielitzki *et al* 1990), offers considerable promise for determining endogenous ileal N flow, though the method may not be suitable for the determination of endogenous ileal amino acid flows (Souffrant *et al* 1986).

The ^{15}N-isotope dilution method relies on the assumtion that the labelling of endogenous nitrogen is similar to that of the designated precursor N-pool. This means that the ^{15}N-enrichment of the precursor is at a similar level to that of the endogenous protein (N x 6.25)

mixture being excreted from the digestive tract. On theoretical grounds the direct precursor pool for endogenous ileal protein secretion is the weighted sum of the intracellular free, tRNA bound or secretory protein bound N-pools in gut epithelial, pancreatic acinar tissue and from the other tissues involved in the endogenous protein secretion. Although strictly part of the "non-dietary" component rather than the "endogenous" component, the contribution of the small intestinal microflora should also be taken into account. During the development of the ^{15}N-isotope dilution method the TCA-soluble fraction of blood plasma, which should be in equilibrium with the direct precursor pools, has been adopted as the precursor N-pool for synthesized endogenous N excreted at the terminal ileum (Souffrant *et al* 1981, 1986, 1993).

A further basic requirement of the ^{15}N-isotope dilution technique is the establishment of steady-state conditions. A steady-state is achieved when the level of ^{15}N-enrichment in the N-pool does not change substantially over time. In the present study the estimated ^{15}N-enrichment of the total N-pool of TCA-soluble blood plasma and ileal digesta remained fairly constant during days 8, 9, and 10 of the infusion period (Table 4). Calculation of the ^{15}N-enrichment time-course of the TCA-soluble blood plasma (Figure 1) showed similar values after 5 days of infusion. Souffrant *et al* (1993), using a continuous i.v. infusion of ^{15}N-leucine at a daily rate of 40 mg/kg bodyweight, reported similar ^{15}N-enrichment time-course for TCA-soluble plasma. Steady state conditions in the precursor pool were attained in the present study after a few days of infusion and certainly before collection of ileal digesta on days 8, 9 and 10.

In several studies where highly digestible diets have been fed to simple-stomached animals (Souffrant *et al* 1981, 1986, 1993), dilution factors close to unity have been found with the ^{15}N-isotope dilution method. Given that the dietary protein from various sources have been almost completely absorbed, this give extra confidence in the use of the ^{15}N-isotope dilution method. Contrary to this, is the finding of Moughan *et al* (1992) using tritiated leucine labelled rats. In the present study with EHC, the dilution factor was high (72%) but significantly lower than unity ($P < 0.05$). This indicates that the N found in ileal digesta also contained undigested dietary nitrogen and/or that the "non-dietary" proteins of the ileal digesta were not uniformally labelled in relation to the TCA-soluble plasma precursor pool. A critical assumption of the ^{15}N-method is that the specific activity of the TCA-soluble pool equals the specific activity found in the non-dietary protein present in the gut lumen. It is not clear from the literature as to wether this assumption is correct. The aim of the present study was to compare estimates of endogenous ileal protein loss determined using the ^{15}N-isotope dilution method, where the latter inherent assumption is made, with endogenous protein excretion determined by an independent baseline method.

Recently, a new approach, the peptide alimentation method, for determining endogenous

ileal amino acid flow has been proposed by Moughan *et al* (1990). Although the method is not suitable for general practical application (the method cannot be applied to plant foods containing fiber and anti nutritional factors), the approach is direct and should provide meaningful amino acid flow estimates at least for pigs fed animal protein sources. The peptide alimentation method was used as a baseline in the present study, to evaluate the ^{15}N-isotope dilution approach.

The peptide alimentation method has been applied to determine endogenous nitrogen and amino acid losses in several studies (Moughan and Rutherfurd 1990; Butts *et al* 1991; Moughan *et al* 1992; Butts *et al* 1993a). In this method, the animal is fed an enzymic hydrolysate of casein as the only source of dietary nitrogen. These dietary peptides and free amino acids represent the products of *in vivo* casein digestion, and would thus be expected to behave in a physiologically normal manner within the digestive tract.

The main limitation of the method is that it may underestimate endogenous ileal amino acid excretion to some degree, because endogenous free amino acids and peptides are discarded in the low molecular weight ultrafiltration fraction. There is evidence, however, that the levels of endogenous amino acids and peptides in ileal digesta are low (Moughan *et al* 1990; Moughan and Schuttert 1991; Butts *et al* 1992), which means there is only a small underestimation of endogenous ileal nitrogen and amino acid loss. Also, it is possible that estimates of endogenous loss determined with the peptide alimentation method may be influenced in some way by the enzymic hydrolysate of casein itself, and thus be an artefact of this particular dietary treatment.

There is a possibility that some non-absorbed dietary free amino acids and peptides become part of the endogenous proteins in ileal digesta, which could result in an overestimation of endogenous loss. This could be caused by an inefficiency of the ultrafiltration devices resulting in the inadequate separation of the dietary peptides and free amino acids from the endogenous proteins, or the ionic or covalent bonding of the peptides and amino acids to the endogenous proteins. Butts *et al* (1991) evaluated the efficiency of the ultrafiltration devices by determining the recovery of nitrogen in the ultrafiltration (mol wt exclusion limit of 10,000 Da) fractions of a range of purified protein, peptide and amino acid solutions. An effective filtration (generally > 90% separation) of these substrates based on their nominal molecular weight was found. The binding of free amino acids and peptides to plasma proteins has been reported to occure in blood (Williams *et al* 1972; Ohara and Ariyoshi 1979). The is evidence from recent studies, however, that show indirectly that this does not occure in digesta. Butts *et al* (1993a) found that the endogenous ileal amino acid flows for pigs fed a synthetic amino acid diet were not higher than those for protein-free fed pigs, indicating that the dietary free amino acids were not bound-to or trapped-in the endogenous proteins. A study to directly

investigate the binding of casein peptides and free amino acids (C A Butts and P J Moughan, unpublished) by adding EHC to rat ileal digesta prior to ultrafiltration, found an almost complete recovery of the added EHC free amino acids and peptides. Furthermore, the endogenous ileal amino acid excretion determined under peptide alimentation is similar to that determined using diets containing protein (Moughan and Rutherfurd 1990; Moughan and Rutherfurd 1991; Butts *et al* 1993a). Use of the peptide alimentation ultrafiltration technique as a base-line method for the evaluation of the ^{15}N-isotope dilution technique with hydrolyzed casein-fed pigs appears to be justificable.

The mean endogenous AA flows for the EHC-fed pigs in the present study (Table 2) were similar to those reported by Moughan *et al* (1992) and Butts *et al* (1993a,b). In addition, Dary-Vrillon *et al* (1991) and Chung and Baker (1992) reported similar ileal AA flows when feeding a casein based diet to pigs. The AA composition of endogenous dry matter at the terminal ileum, determined in the present study, indicates a preponderance of non-essential AA (Glu, Asp, Pro, and Ser). Glutaminc acid, in particular, was present at a high concentration. This was also observed by other workers (Darcy-Vrillon *et al* 1991; Chung and Baker 1992; Moughan *et al* 1992; Butts *et al* 1993a,b). Glycine, Pro, threonine (Thr), arginine (Arg), Glu, and Asp are the predominant components of mucus glucoproteins (Bella and Kim 1972; Cetta *et al* 1972; Allen 1981; Dekker 1990). Because glycoproteins are particularly resistant to acid and enzymatic digestion (Hashimoto *et al* 1973; Hoskins 1978; Dekker 1990) it can be concluded that mucus forms a major source of ileal endogenous AA and N flow. Findings by Moughan and Schuttert (1991), Butts *et al* (1993a,b) and Lien *et al* (1993) support this conclusion. The total AA nitrogen (2.67 g/kg dry matter intake) of the treated digesta made up 73% of the total N of the treated digesta. This finding is similar to the results reported by Butts *et al* (1993a).

The mean dilution factor found in the present study for the EHC diet was 0.72. This is only slightly outside the expected range for the dilution factor (0.74 to 1.0) for EHC-protein, according to Moughan *et al* (1992). Ileal endogenous N flow determined with the ^{15}N-isotope dilution was numerically lower than that determined by the peptide alimentation, ultrafiltration method which may have been caused by the inclusion of mucosa glucoproteins and bacterial N in the determination of the endogenous ileal N flow. Whereas it can be assumed that mucosal and bacterial proteins are completely included in the processed digesta of the peptide alimentation, ultrafiltration method, its inclusion in the endogenous ileal N flow determined with the ^{15}N-isotope dilution method depends on the ^{15}N-labelling. However, a substantial proportion of the maintenance of the intestinal mucosa and the synthesis of secreted proteins occurs at the expense of luminal, dietary amino acids (Alpers 1987; De Lange *et al* 1992). Bacteria will use endogenous urea or ammonia to synthesize microbial protein. It is not known,

however, to what extent the incorporation of urea or ammonia N into microbial protein occurs in the small intestine.

The present findings of the relative high dilution factor for pigs fed an EHC based diet and that the determined endogenous flows were not significantly different between the two techniques studied, provides some support for the ^{15}N approach. The latter technique may be particular useful for the determination of relative differences in endogenous ileal N flows in the pig. The present study, however, only incorporated one dietary nitrogen source and more work is required before definite conclusions can be made concerning the validity of the ^{15}N technique.

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Chapter 3

Effect of Level of Dietary Neutral Detergent Fiber on Ileal Apparent Digestibility and Ileal Nitrogen Losses in Pigs

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ABSTRACT: A study was conducted with 20 barrows (average BW 25 kg) to determine the effect of various levels of neutral detergent fiber (NDF) in the diet on the apparent ileal nutrient digestibility, ileal diaminopimelic acid (DAPA) content, and consequently the amount of ileal endogenous nitrogen. The pigs were fitted with a post-valvular T cecal cannula. They were fed .8 kg/d of a corn starch-based semisynthetic diet formulated to contain equal amounts of protein and starch and 0, 60, 120, or 180 g of purified NDF/kg of diet, included at the expense of glucose. The purified NDF (pNDF) was isolated from wheat bran using an incubation procedure with pancreatin. Ileal digestibility of NDF was approximately 17%, and was independent of the pNDF level in the diet. By increasing the amount of pNDF in the diets, apparent ileal digestibilities of dry matter (DM), nitrogen (N), neutral detergent insoluble N (NDF-N), and ash decreased linearly ($P < .05$). The DAPA content of the ileal digesta (g/d) was not affected by the percentage of pNDF in the diets. Calculation of the ileal bacterial N excretion indicated that more than 50% of the ileal N was of bacterial origin. With increased percentage of dietary pNDF, both endogenous and exogenous N in ileal chyme were linearly increased ($P < .05$). Thus an increase in the dietary fiber content lead to a decreased apparent ileal protein digestibility due to increased ileal losses of both endogenous and exogenous protein.

Key Words: Pigs, Fiber, Digestibility, Diaminopimelic Acid, Endogenous Protein

Introduction

Dietary fiber is a heterogeneous mixture of structural (cellulose, hemicellulose, and pectin) and non-structural (gums, mucilages, and algal types) polysaccharides and lignin (Low, 1985a; Englyst, 1989; Potkins et al., 1991). Several investigators (e.g., Low, 1982; Graham et al., 1986; Fernandez and Jφrgensen, 1986; Graham and Åman, 1987a,b) reported that addition of fiber to the diet can lead to a lower apparent ileal digestibility of starch, crude protein, fat, and minerals. This can result from changes in the rate of absorption of the different nutrients

(Vahony and Cassidy, 1985; Low, 1985b; Rerat, 1985), and(or) from differences in endogenous N excretion (Sauer et al., 1977; Taverner et al., 1981; Green et al., 1987). According to Sauer and Ozimek (1986), the level and source of dietary fiber are the two most important factors influencing the amount of endogenous nitrogen (N) and of amino acids present in the ileal digesta.

Most natural fiber sources contain a large number of different fibrous components. Each type of fiber has its own composition. This means that structural and physical characteristics are specific for each type. In addition, each type of fiber can have its own specific interaction with other dietary components (Laplace et al., 1989). In practical diets, it is therefore impossible to predict the contribution from each of the dietary fibers or fiber components on the amount of N passing the terminal ileum.

In the present investigation, an isolated complex of water insoluble, neutral detergent fiber was prepared from wheat bran and further purified. The effect of different levels of this purified NDF in the diet on the ileal passage of nutrients, including N (both endogenous and exogenous N) was determined and compared with a NDF-free diet.

Materials and Methods

Preparation of Fiber. The purified NDF (pNDF) was prepared using the following procedures (Mollee et al., personal communication): a) wheat bran was ground through a 1.5-mm mesh screen (Urschel Cutting Mill, Fr. Urschel, Germany), b) nylon bags (50-cm x 50-cm; 200 μm mesh size) were filled with 1 kg of ground wheat bran, c) wheat bran was washed for 90 min (maximum 85°C, 45 min) using a commercial washing machine with addition of 100 g of soap (Teepol HB 7, Shell Nederland Chemie B.V.), d) after removing the soap by repeated rinsing (three times) with cold water and centrifugation, the remaining substrate (around .5 kg) was incubated with 2.5 L of a 1.5% pancreatin solution (Merck Nr.7133) for 24 h at 30°C in a sealed container, e) the substrate was washed with cold water and then the washing procedure of step c was repeated, f) the washed and centrifuged pNDF was dried for 72 h at 70°C. The pNDF was stored at room temperature in a sealed container.

Animals and Diets. Twenty 10-wk-old barrows (Large White X Landrace) were fitted with a post-valvular T caecum cannula (PVTC) as described by Van Leeuwen et al. (1991). The animals were placed in individual cages. Following a 2-wk adaptation period, the animals were weighed and were than assigned randomly to one of four treatments. The average initial BW of the animals at assignment was 21.9 kg and the average final BW was 27.4 kg. All pigs

received .8 kg/d of the experimental diet. The composition of the four experimental diets is given in Table 1. All diets contained equal amounts of protein and starch and contained 0, 60, 120, or 180 g of the pNDF/kg of diet. The pNDF replaced glucose in the diets on a dry matter basis. Water was freely available at all times from a low- pressure drinking nipple.

TABLE 1. Composition and chemical analyses of the experimental diets (as-fed basis)

	Diet			
	1	2	3	4
Ingredients, g/kg				
Soya isolate	220.0	220.0	220.0	220.0
Cornstarch	493.5	493.5	493.5	493.5
Soya bean oil	20.0	20.0	20.0	20.0
Glucose[a]	200.0	140.0	80.0	20.0
Purified NDF	-	60.0	120.0	180.0
Vitamin/mineral mixture[b]	100.0	100.0	100.0	100.0
$CaCO_3$	13.5	13.5	13.5	13.5
$CaHPOH_2O$	21.0	21.0	21.0	21.0
NaCl	5.0	5.0	5.0	5.0
$KHCO_3$	6.0	6.0	6.0	6.0
$NaHCO_3$	4.0	4.0	4.0	4.0
DL-Methionine	2.0	2.0	2.0	2.0
L-Threonine	1.0	1.0	1.0	1.0
Cr_2O_3	4.0	4.0	4.0	4.0
Dry matter, g/kg of diet	913.6	918.8	910.6	913.2
Chemical compositon as analyzed, g/kg (DM basis)				
CP (N x 6.25)	214.8	223.5	221.7	221.1
Crude fiber	5.7	24.1	34.0	50.4
Ash	52.8	55.0	58.5	59.8
NDF	3.3	60.9	120.8	176.5
ADF	3.3	21.9	39.5	63.5
ADL[c]	0.9	7.7	13.2	19.7

[a] *Meritose EF (monohydrate: 92% glucose and 8% water; Fr. CN Schmidt, Amsterdam, The Netherlands).*

[b] The vitamin/mineral mix provided the following per kilogram of feed: 9,000 IU of vitamin A; 1,800 IU of vitamin D_3; 40 mg of vitamin E; 1.36 mg of menadione as dimethyl-pyrimidinol bisulfite; 5 mg of riboflavin; 40 µg of cobalamine; 30 mg of niacin; 15 mg of d-pantothenic acid; 120 mg of choline chloride; 50 mg of ascorbic acid; 2 mg of thiamin; 3 mg of pyridoxine; .1 mg of d-biotin; 1 mg of folic acid; .38 mg of K (KI); .525 mg of Co ($CoSO_4$); .06 mg of Se (Na_2SeO_3); 80 mg of Fe ($FeSO_4$); 254 mg of Cu ($CuSO_4$); 44 mg of Mn (MnO_2); 72.8 mg of Zn ($ZnSO_4$); 40 mg of tylosin.

[c] Acid detergent lignin.

Collection of Digesta Samples. After an adaptation period of 6 d on the experimental diets, ileal digesta was collected for 24 h on d 7, 9, and 11. Ileal digesta were collected directly into a bag fixed to the cannula. The plastic bags were removed every 30 min, weighed, and immediately stored at -20°C.

Apparent Ileal Digestibility. The apparent ileal digestibility coefficients for DM, N, NDF, and ash were determined by the chromic oxide ratio method. The ileal digestibility of N bound to neutral detergent fiber (NDF-N) was calculated from the amount of daily ingested NDF-N bound to the pNDF and the daily excreted amount of NDF-N. The NDF-N in ileal chyme was determined from one pooled (across pigs and days) sample of ileal digesta per treatment group. The method for the determination of NDF-N involved the isolation of the neutral detergent residue (NDR) following the analytical procedure for NDF, except the final ashing step, followed by the determination of the N content in the NDR.

Ileal Bacterial Nitrogen. The amount of ileal bacterial N was estimated by measuring the diaminopimelic acid (DAPA) content in the ileal digesta of the pigs fed different levels of pNDF in the diet. The ileal bacterial N content was calculated by using the value of 26.4 mg of DAPA/g of bacterial N according to Wünsche et al. (1991).

Estimation of Ileal Endogenous and Exogenous Nitrogen. Glucose is highly digestible, and was assumed to be completely absorbed at the end of the duodenum. Therefore, effects of glucose on the excretion of endogenous N losses are assumed to be negligible. Because of the constant intake of N and DM from the basal diet it can be expected that the basal ileal endogenous and exogenous N secretion is also rather constant. Therefore, changes in the ileal excretion of N are assumed to be affected only by the incremental addition of pNDF in the diets. The amount of ileally excreted N (g/d) affected by the added pNDF in the experimental diet groups (Diet 2 to 4) was calculated according to the following equation:

$$\mathbf{NA = NI - NB} \qquad [1]$$

where NA = the additional N, NI = the N excretion of the experimental diet groups (Diet 2 to 4), and NB = the mean basal N excretion determined with the basal diet group (Diet 1). The NA probably consists of both endogenous and exogenous N.

The aim of the isolation procedure of the pNDF from the wheat bran was to minimize enclosed nutrients without damaging the structure of the fibrous material. However, these intact fibrous structures in the cell wall may still enclose some cell contents, consisting of starch,

protein and other nutrients. These components cannot be reached by enzymes and thus cannot be hydrolyzed nor digested (Graham and Åman, 1987a). Consequently, they form a source of exogenous losses at the end of the ileum. Thus, part of the NA passing the terminal ileum is NDF-N. The amount of additional Non NDF-N in ileal chyme (NAE), therefore, was calculated by subtracting the determined NDF-N from NA as:

$$\mathbf{NAE = NA - NDF\text{-}N} \qquad [2]$$

According to the definition of NA and combining Equations [1] and [2], NAE can be expressed as:

$$\mathbf{NAE = (NI - NB) - NDF\text{-}N} \qquad [3]$$

Thus at least part of nitrogen passing the terminal ileum is NDF-N. This can also be derived from amino acid analyses of NDF-N in ileal chyme. The amino acid composition was similar to that of amino acid composition of feed NDF-N (Schulze, 1994; unpublished).

Analytical and Statistical Procedures. Prior to chemical analyses, digesta samples were freeze-dried and ground through a 1-mm mesh screen. Daily samples of ileal digesta were pooled for each animal before chemical analysis. The analysis of DM, ash, and N in feed and ileal digesta were carried out according to AOAC procedures (1975, 1984). The crude fiber content in feed was analyzed as described by Standards of the Netherlands Normalization Institute (1985). Crude fat and starch contents were estimated according to the procedure described by Huisman (1990) and Åman and Hesselman (1984), respectively. Analysis of the NDF and ADF content were carried out according to the methods described by Huisman (1990), whereas acid detergent lignin (ADL) was analyzed using the methods described by Goering and Van Soest (1970). The chromic oxide content was determined by the method described by Bosch et al (1988). Diaminopimelic acid analyses were carried out according to Ahrens and Kaufman (1985). All chemical analyses were performed in duplicate.

Statistical analyses of the data was performed using the GLM procedure of SAS (1990). Linear, quadratic, and cubic effect of dietary pNDF levels were evaluated with single degree of freedom comparisons appropriate for equally spaced treatments (orthogonal polynomials) according to procedures outlined by Snedecor and Cochran (1989).

Results

The Purified Neutral Detergent Fiber. The nutrient composition of the wheat bran and the purified NDF are given in Table 2. The pNDF contained 910.9 g of NDF/kg of DM. This pNDF contains all major components (hemicellulose, cellulose, and lignin) of plant cell walls, except water soluble components. The concentrations of crude fiber and NDF were increased 98 and 86%, respectively, in the pNDF compared with wheat bran. The concentrations of N and starch were reduced by 71 and 77%, respectively, in the pNDF compared with the wheat bran, whereas the crude fat content was approximately the same.

TABLE 2. Analyzed chemical composition (g/kg of DM) of wheat bran and the purified NDF

Item	Wheat bran	purified NDF
Dry matter, g/kg of product	888.2	917.0
Nitrogen	29.0	8.4
Starch	179.0	41.4
Crude fat	42.8	50.2
Ash	65.7	31.3
Crude fiber	130.6	258.5
NDF	490.0	910.9
ADF	150.0[a]	337.0
Acid detergent lignin	30.0[a]	89.4

[a] Taken from United States- Canadian Table of Feed Composition (1982).

Apparent Ileal Digestibilities. Increasing the pNDF concentration in semisynthetic diets linearly decreased ($P < .002$) the apparent ileal digestibility of DM, N, NDF-N, and ash (Table 3). The ileal digestibility of NDF was not affected ($P = .8$) by pNDF in the diet.

Content of Diaminopimelic Acid and Ileal Bacterial N. The contents of ileal DAPA and bacterial N content (mg/d and g/d, respectively) were not affected ($P > .30$) by pNDF in the diet (Table 4). The ratio between the content of ileal DAPA and total N and the ratio between bacterial N and total N content passing the terminal ileum also were not affected ($P > .30$) by the dietary treatment (Table 4).

TABLE 3. Apparent ileal digestibility (%) of DM, N, NDF-N, NDF, and ash, in cannulated pigs fed diets with different levels of purified NDF

	purified NDF, g/kg					P - value	
Item	0	60	120	180	SEM	L[a]	Q[a]
DM	92.0	85.7	78.5	72.1	.57	.001	.968
N	88.9	88.4	86.2	84.0	.59	.001	.188
NDF-N[b]		57.1	60.0	62.7	1.03	.002	.910
NDF		17.0	16.2	18.0	2.78	.801	.707
Ash	53.4	46.7	35.2	28.1	1.65	.001	.921

[a] L = linear effect; Q = quadratic effect.
[b] Nitrogen bound to the neutral detergent fiber.

TABLE 4. The daily amount of diaminopimelic acid (DAPA) and bacterial N excretion, and their relation to the total amount of N per day in the ileal digesta of cannulated pigs fed on diets with various levels of purified NDF

	purified NDF, g/kg					P - value	
Item	0	60	120	180	SEM	L[a]	Q[a]
DAPA; mg/d	48.30	53.79	48.24	58.48	9.67	.572	.809
DAPA/total N[b]	.017	.017	.014	.014	.003	.330	.853
Bacterial N[c]; g/d	1.830	2.037	1.827	2.215	.366	.572	.809
Bacterial N/total N[d]	.655	.647	.514	.544	.104	.330	.853

[a] L =linear effect; Q = quadratic effect.
[b] Gram of DAPA/g of ileal total N per day.
[c] Bacterial N contains 26.4 mg DAPA per g of bacterial N.
[d] Gram of bacterial N/g of total ileal N per day.

Additional Ileal N Excretion. The amount of ileal excreted N, NA, NDF-N, and NAE increased linearly ($P < .05$) as the percentage of pNDF increased in the diet (Table 5).

Table 5. Differentiation of ileal N losses (g/d) into additional total N (NA), additional exogenous (NDF-N) and additional Non NDF-N (NAE) excretion of N as affected by various levels of purified NDF in the diets

	purified NDF, g/kg					P - value	
Item	0	60	120	180	SEM	L[a]	Q[a]
Ileal N, g/d							
Total excretion	2.787	3.041	3.563	4.129	.152	.001	.321
NA[b]	-	.255	.776	1.342	.169	.001	.916
NDF-N[c]	-	.198	.369	.518	.010	.001	.441
NAE[d]	-	.056	.407	.824	.162	.006	.867

[a] L = linear effect; Q = quadratic effect.
[b] Calculated from the means per treatment according to Equation [1] (NA [extra total N excretion] = NI - NB).
[c] Nitrogen bound to the neutral detergent fiber.
[d] Calculated according to Equation [3] (NAE = [NI - NB] - NDF-N).

Discussion

Purified Neutral Detergent Fiber and Ileal Apparent Digestion. The decrease in the ileal digestibility of DM can be seen as a direct result of replacing a highly digestible carbohydrate source (glucose) with one of lower digestibility (pNDF). Sauer et al. (1991) found similar results on ileal digestibility of DM by replacing cornstarch with Alphafloc or straw. Such an effect of wheat bran diets was also demonstrated by Newton et al. (1983).

The increase of the ash content in the diets (Table 1) is associated with the ash content in the pNDF as shown in Table 2. This high amount of ash in the pNDF and in the ileal digesta of the animals fed increasing amounts of pNDF may be explained by the binding of the dipole of the water molecule to cations and anions of mineral salts and also by increased water binding capacity by fibrous compounds (Bergner, 1986). In addition the cation exchange capacity of the fiber may be a causative factor as outlined by Van Soest et al. (1991).

Our results show that almost 20% of the ingested NDF is digested before the end of the ileum. Because pigs do not posses enzymes to hydrolyze NDF, this observed digestion must be the result of bacterial fermentation. Because NDF digestibility values were observed to be independent of the NDF inclusion level, there appears to be sufficient bacterial activity in these young pigs at all NDF levels used in this experiment. Fermentation of NDF before the end of the ileum also has been observed by other researchers. Laplace et al. (1989) found that the ileal digestibility values of the NDF fraction in pigs fed diets supplemented with soya bean hulls, wheat bran or a combination of both were 15%, 6%, and 9%, respectively. Buraczewska et al.

(1988) showed that the degree of fermentation of NDF in the small intestine varied between 10% to 32%, depending on the source of NDF. According to Drochner (1984), Graham et al. (1985), Longland et al. (1988), and Buraczewska et al. (1988) a considerable part of NDF, probably the hemicellulose fraction, may be fermented in the small intestine. Chesson et al. (1985) found cellulolytic activity in chyme of the small intestine of pigs.

Purified Neutral Detergent Fiber and Ileal Nitrogen Losses. The effect of increasing amounts of pNDF in the diets on the increasing amount of N passing the terminal ileum can be explained in various ways.

1) Protein in the pNDF is only partially digested because the digestive enzymes have limited access to the cell wall components as well as the cell contents enclosed by them (Bjergegaard et al., 1991; Shah et al., 1982). Our results of ileal digestibility of N in NDF agree with those of Graham et al. (1986). These authors reported an apparent ileal digestibility of CP in wheat bran of 63.3% in pigs. It also has been reported in humans that the faecal N digestibility of various wheat brans ranges from 62.8% to 73.0% (Saunders, 1980). From our results, we conclude that with increasing amounts of pNDF in the diet the passage of undigested N enclosed in or associated with N in fiber increased. This, however, only partly explains the additional total N excretion at the end of the ileum with increased levels of pNDF in the diet.

2) There also may be an increase in endogenous N passing the terminal ileum with increasing pNDF levels in the diet. Increased losses of endogenous N can be explained by: a) an increased secretion, b) a decreased re-absorption, or c) both. It has been reported that endogenous N secretions including pancreatic juice (Ikegami et al. 1990), bile (Portman et al., 1985), mucus (Low, 1989) and sloughed off epithelial cells (Shah et al., 1982) are secreted in larger amounts when experimental animals are fed purified diets supplemented with fiber. Langlois et al. (1987) showed that the inclusion of 40% wheat bran in the diet of pigs increased the secretion of pancreatic juice and protein. Fiber may also absorb amino acids and peptides and withhold them from absorption (Bergner et al., 1975; Sauer et al., 1991). Moreover, the water-binding capacity of the fiber was found to reduce the diffusion of the products of digestion towards the mucosal surface (Dierick et al., 1989).

According to these reported characteristics of fiber, the observed increased N flow at the terminal ileum as a result of the increased pNDF in the diets may be a combination of an increased amount of endogenous N and a decreased absorption of exogenous N. From determination of NDF-N in ileal chyme and the amino acid composition (Schulze, 1994; unpublished) it can be derived that at least part of increased ileal N is caused by the diet. The rest of the increase is than due to additional exogenous and endogenous N. In the studies of Sauer et al. (1977) and Taverner et al. (1981) the ileal endogenous protein output increased

with the dietary fiber level up to approximately 100 g of NDF/kg of diet and not with further increases. Recent results by De Lange et al. (1989) and Furuya and Kaji (1992) also showed that increases in levels of dietary fiber did not give additional ileal endogenous flow of N. Some authors have used various amounts of purified fibrous constituents such as purified wood cellulose or pectins. De Lange et al. (1989), Furuya and Kaji (1992), and Leterme et al. (1992) clearly showed that with purified wood cellulose as the dietary fiber source, no effects on the ileal endogenous N excretion in pigs were observed. However, the addition of pectins to the diet of pigs increased the endogenous N at the terminal ileum (De Lange et al., 1989; Mosenthin et al. , 1989). Because we used purified NDF the effect of pectin was not a contributing factor.

Our data and the above mentioned literature indicate that NAE consists mostly of additional endogenous N. In explaining the additional ileal endogenous N with increased amounts of dietary NDF in the present experiment, there are at least two possibilities. The fibrous constituents, other than cellulose, in the pNDF [e.g. hemicelluloses and(or) lignins] have induced the increased endogenous N secretion. Secondly, the intact fibrous structure of the pNDF may be responsible for an increased secretion and a decreased reabsorption of endogenous N in the small intestine.

3) In the present experiment, no effects of incremental addition of pNDF in the diets on ileal bacterial growth were found. To measure the ileal flow of bacterial N, DAPA was used as a marker. The DAPA is present in bacterial cell wall mucoprotein but is not found in plant or animal cells (Rowan et al., 1992). Its concentration, however, may vary considerably among different species of bacteria (Czerkawski,1974). Furthermore Dufva et al. (1982) and Laplace et al. (1985) concluded that bacterial N may change with changes in microbial populations. The present data of ileal DAPA are in agreement with those of Rowan et al. (1992), with pigs of similar body weight. However, the DAPA content was found to vary with the technique of collecting ileal chyme. Animals with ileo-rectal anastomoses with closed colon (IRAg) showed much lower values than animals with re-entrant ileo-caecal cannulae (Wünsche et al., 1991). The present calculated bacterial N proportion of the total N in the ileal digesta are in agreement with those of Wünsche et al. (1991), but are higher than values reported by Drochner (1984; ranging from 23% to 32%). The reason that we found no effect of increasing levels of pNDF in the diet on bacterial growth may thus be related to the method. Moreover there may be a limited bacterial activity in young animals compared to older animals.

In a following study, the ileal endogenous excretion of N will be evaluated using the ^{15}N isotope dilution technique. Additional research should be carried out that will focus on a) the dietary fibrous factors affecting the increase of ileal endogenous protein excretion and b) the validity of using the NDF content in a feed for correction of the apparent digestibility of N.

Implications

From the results of our study, it can be concluded that the ileal nitrogen excretion in pigs will increase with increasing amounts of a purified neutral detergent fiber concentrate in the diet. This will partly be of exogenous and endogenous origin. Thus inclusion of dietary fiber will increase the nitrogen content of ileal and probably the fecal matter as well. The degree of increase is dependent on dietary fiber level and on the composition of the fiber fraction. Adding fiber to pigs diets may impact nitrogen utilization in the animal and ammonia volatilization in the facilities. The results of the study here refer to wheat bran fiber because wheat is an important feedstuff.

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Chapter 4

Dietary Level and Source of Neutral Detergent Fiber and Ileal Endogenous Nitrogen Flow in Pigs

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Abstract: Two experiments were conducted to study the effect of dietary level and source of neutral detergent fiber (NDF) on ileal dry matter flow and on total and endogenous N flow at the terminal ileum. Twenty-two crossbred 6-wk-old castrated male pigs with an average BW of 9 kg were used. The pigs were fitted with a post-valvular T-cecal cannula and two indwelling blood catheters. During the experimental period of 10-d, the pigs were 11 wk of age with an average BW of 14 kg. They were fed 2.6 times their maintenance requirement for energy of a corn starch-based semisynthetic diet. The diets contained 178 g of soya isolate/kg as the only source of nitrogen (N). In diets of Exp. 1, purified NDF was included at 0 and 200 g/kg of feed at the expense of glucose. The diets of Exp. 2 contained one of the three different NDF sources (purified NDF, wheat bran, or sunflower hulls) at a level of 144 g of NDF/kg of DM. The purified NDF (pNDF) was isolated from the same batch as the wheat bran (WB) used in Exp. 2. The endogenous N flow at the terminal ileum of these pigs was determined with the ^{15}N-isotope dilution method. The inclusion of NDF in the experimental diets increased ($P < .05$) the daily DM flow at the terminal ileum. Dry matter flow was increased .697 g for every g/kg increase in NDF in the diet. The different sources of dietary NDF gave similar ($P > .05$) ileal DM flows. The inclusion of pNDF (Exp. 1) increased ($P < .05$) the ileal flow of total N by 1.884 g/kg of dietary dry matter intake. This increase is composed of 59% endogenous and 41% exogenous N. With the inclusion of various NDF sources (Exp. 2) similar ($P > .05$) amounts of endogenous N passing the terminal ileum were obtained. Calculated from results of Exp. 1 and 2, endogenous ileal N flow was increased .008 g for every g/kg increase in NDF in the diet. The total ileal N flow, however, varied ($P < .05$) with the inclusion of various NDF sources in the diets (Exp. 2). The results show that the increase in ileal N with dietary NDF depends on level and specific NDF.

Key Words: Pig, Neutral Detergent Fiber, Ileal Dry Matter, Ileal Endogenous Nitrogen, Isotope Dilution Method

Introduction

Dietary fiber can increase the excretion of nitrogen (N) at the terminal ileum of nonruminant animals (Potkins et al., 1991; Sauer et al., 1991). Part of the increase is related to the secretion of endogenous nitrogenous substances into the gastrointestinal tract (Low, 1989). Dietary fiber, by definition, embraces a wide spectrum of components (Southgate and Engelyst, 1985). Therefore, a combination of the effects of various purified dietary fibers, estimated separately, may not have the same effect on ileal N flow as a natural dietary fiber of similar composition. A systematic approach to this problem requires the inclusion of some form of a single chemical or physical property of the dietary fiber, and the study of the effect of this property per se on the passage of N at the terminal ileum, as proposed by Larsen et al. (1993). In order to study these single properties, purified fibrous fractions are required. Therefore, it is important to analyze and specify this fraction, because dietary fiber is still essentially defined by the method used for analyses and(or) isolation (Graham and Åman, 1991).

The present study aimed to investigate N and DM flow at the terminal ileum in pigs fed different dietary sources of NDF - wheat bran and sunflower hulls compared to NDF-free and isolated wheat bran NDF. The influence of level and source of dietary NDF on endogenous N flow at the terminal ileum in these pigs was determined with the ^{15}N-isotope dilution method.

Materials and Methods

General: Animals and Housing. Two experiments were conducted using crossbred (Dutch Landrace x Yorkshire) castrated male pigs with an age of approximately 5 and 6-wk for Exp. 1 and 2, respectively. They were obtained from a commercial breeding farm. The animals were individually housed in 1.2-m x 1.2-m transparent smooth-walled metabolism cages and allotted randomly to one of the dietary treatment groups. The environmental temperature was maintained in a range of 23 to 26°C and the relative humidity was kept at 50 to 70%. Ethics approval for this study was given by the TNO-Institute for Nutrition and Food Research and Wageningen Agricultural University Animal Ethics Committees.

Diets and Feeding. During the preliminary period, the pigs were fed a commercial diet containing 180 g of CP/kg of diet. The diet was given twice daily and approximately 150 g was given each time during the period before the ileal surgery, at 0800 and 2000, respectively. After surgery, the pigs were fed increasing amounts of the various experimental diets (Table

1), until they consumed 2.6 times maintenance requirement for energy (ARC, 1981). The diets contained approximately 9.2 MJ of net energy per kg. The experimental diets were fed four times a day in similar amounts, at 0600, 1200, 1800, and 2400, respectively. Chromic oxide was included in the diets at 1 g/kg as an indigestible marker. The experimental diets of Exp. 1 and 2, respectively, differ only in the level and source of dietary NDF included at the expense of glucose and cornstarch. The diets were mixed with water (1:2, wt/vol) immediately before feeding and fresh water was available for 30 min after each meal.

Experiment 1. The first experiment was conducted using 10 pigs with an average initial (± SE) BW of 8.8 kg (± .31). The average BW of the animals at assignment, at the beginning of feeding the experimental diets, was 11.1 kg (± .29). At the end of the experiment, the average BW was 15.3 kg (± .40). Experiment 1 was conducted to study the effect of a high level of dietary NDF on the N flow at the terminal ileum compared with a NDF-free diet. This study focused on water insoluble fiber. For that purpose, NDF was purified from wheat bran according to the procedure described by Schulze et al. (1994). The experimental diets contained equal amounts of protein and starch and contained 0 (Diet NDF-free) or 200 g (Diet pNDF1) of the purified NDF (pNDF)/kg of diet. The pNDF replaced glucose in the diets on a dry matter basis.

Experiment 2. Twelve pigs, initially weighing 9.6 kg BW (± .21), were used to determine the effect of different dietary sources of NDF on the excretion of ileal N. Different fiber sources were included in the experimental diets at the expense of glucose. These sources were pNDF (pNDF2), wheat bran (WB), and sunflower hulls (SFH). These fibers were water insoluble fiber sources as defined by NDF analyses. Different concentration of pNDF2, WB, and SFH are added in the diets to supply the same level of NDF. The pNDF used in both trials of this study was prepared in one time from one batch of wheat bran used in Diet WB, and also was used in the study of Schulze et al. (1994). The average BW of the animals at assignment, at the beginning of feeding the experimental diets, was 13.4 kg (± .21). At the end of the experiment, the average BW was 17.5 kg (± .68).

TABLE 1. Composition and chemical analyses of the experimental diets (g/kg, as-fed basis)

	Experiment 1		Experiment 2		
Item	NDF-free[a]	pNDF1[a]	pNDF2[a]	WB[a]	SFH[a]
Ingredient					
Soya isolate	178.0	178.0	178.0	178.0	178.0
Corn starch	476.5	476.5	476.5	326.5	476.5
Glucose[b]	250.0	50.0	95.0	100.0	52.0
Purified NDF	-	200.0	155.0	-	-
Wheat bran	-	-	-	300.0	-
Sunflower hulls	-	-	-	-	198.0
Soyabean oil	20.0	20.0	20.0	20.0	20.0
Vitamin/mineral Mix[c]	20.5	20.5	20.5	20.5	20.5
$CaCO_3$	13.5	13.5	13.5	13.5	13.5
CaHPO	21.0	21.0	21.0	21.0	21.0
NaCl	5.0	5.0	5.0	5.0	5.0
$KHCO_3$	6.0	6.0	6.0	6.0	6.0
$NaHCO_3$	4.0	4.0	4.0	4.0	4.0
L-Lysine	2.0	2.0	2.0	2.0	2.0
DL-Methionine	1.9	1.9	1.9	1.9	1.9
L-Threonine	.5	.5	.5	.5	.5
L-Tryptophan	.1	.1	.1	.1	.1
Cr_2O_3	1.0	1.0	1.0	1.0	1.0
DM, g/kg of diet	909.1	914.3	913.6	902.8	906.3
Chemical composition as analyzed, g/kg of DM basis					
Nitrogen	28.6	29.6	29.5	33.8	29.5
NDF	7.7	195.5	142.8	144.7	144.2

[a] NDF-free means basal experimental diet without adding NDF, pNDF1 means basal experimental diet supplemented with 200 g of purified NDF/kg of diet, pNDF2 means basal experimental diet supplemented with 155 g of purified NDF/kg of diet, WB means basal experimental diet supplemented with 300 g of wheat bran/kg of diet, and SFH means basal experimental diet supplemented with 198 g of sunflower hulls/kg of diet.

[b] Meritose EF (monohydrate, 92% glucose and 8% water; Fr. CN Schmidt, Amsterdam, The Netherlands).

[c] The vitamin/mineral mix provided the following per kilogram of feed: 9,000 IU of retinol; 1,800 IU of cholecalciferol; 40 mg of α-tocopherol; 1.36 mg of menadione dimethyl-pyrimidinol bisulfate; 5 mg of riboflavin; 40 µg of cobalamine; 30 mg of niacin; 15 mg of d-pantothenic acid; 120 mg of choline chloride; 50 mg of ascorbic acid; 2 mg of thiamin; 3 mg of pyridoxine; .1 mg of d-(+)biotin; 1 mg of folic acid; .38 mg of K (KI); .525 mg of Co ($CoSO_4$); .06 mg of Se (Na_2SeO_3); 80 mg of Fe ($FeSO_4$); 254 mg of Cu ($CuSO_4$); 44 mg of Mn (MnO_2); 72.8 mg of Zn ($ZnSO_4$); and 40 mg of tylosin.

Experimental Procedure. Both experiments consisted of the periods (Figure 1) used by Huisman et al. (1992). After a period of adaptation to the individual housing in the metabolism cages (8 to 9 d), the pigs were fitted with a post-valvular T-cecal (PVTC) cannula (Van Leeuwen et al., 1991). Two indwelling catheters, one into the external jugular vein (for taking blood samples) and the other into the carotid artery (for the infusion of [^{15}N]-Leucine), were surgically implanted 10-d following the ileal surgery. One day after insertion of the catheters, a constant 10-d [^{15}N]-Leucine infusion was performed at a rate of 5.04 mg of [^{15}N]-Leucine (95% ^{15}N enrichment) kg^{-1} of BW d^{-1}. To minimize the time reaching the steady state of the ^{15}N enrichment in the precursor pool, the animals were primed with .21 mg of [^{15}N]-Leucine/kg of BW. The [^{15}N]-Leucine was dissolved in a sterile non-pyrogenic physiological saline solution. Approximately 50 mL of this solution was daily infused in each animal. Accurate perfusion pumps (B. Braun Melsungen AG, FRG) were used to infuse the solution. To infuse a free-moving pig continuously, the 'Swivel-technique' as described by Van Kleef (1993) was used.

On d 7, 8, and 9 of the experimental period, ileal digesta was collected continuously for 12 h/d from 0800 to 2000 in small plastic bags attached to the cannula that were emptied hourly. The collected digesta were immediately frozen (-20°C).

Blood samples of approximately 5 mL were taken during the infusion time three times each day at 0900, 1500, and 2100. The day before the infusion of the [^{15}N]-Leucine solution, blood was taken to determine the background ^{15}N enrichment of the trichloroacetic acid (TCA)-soluble fraction of the blood plasma. Immediately after sampling, the blood was centrifuged for 10 min at 1,000 x g (De Lange et al., 1990). The plasma from each animal was pooled per day and stored at -20°C. The precipitate was discarded. Before chemical analysis for ^{15}N enrichment, duplicate samples were taken from each sample. Forty percent TCA (.10 mL) was added to .50 mL of the plasma and thoroughly mixed. After overnight (16 h) storage at 4°C, this mixture was centrifuged at 3000 x g for 20 min at 4°C. The resulting precipitate was discarded and the supernatant (approximately .20 mL) was neutralized (pH 7) with 40% NaOH (.040 mL) and subsequently freeze-dried.

Chemical Analyses. Before analyzing, samples of ileal digesta were freeze-dried, ground (< 1 mm), and thoroughly mixed. Total N, dry matter (DM) and chromium were determined in the diet and in the daily samples of freeze-dried ileal digesta. The content of DM and N in the feed and ileal digesta were determined according to AOAC (1984) procedures. Chromium was determined according to Bosch et al. (1989). The content of NDF was determined in the various fiber sources and the experimental diets as described by Huisman (1990). All analyses were performed in duplicate.

For the determination of ^{15}N enrichment in ileal digesta, feed, and TCA-soluble-fraction of blood plasma, individual freeze-dried samples where weighed into special tin capsules (8 mm x 5 mm, Van Loenen Instruments, The Netherlands) so that the N content was approximately 50 ± 10 μg (Van den Berg; personal communication). To determine the ^{15}N enrichment, the samples in the tin capsules were combusted in a "Total Nitrogen Analyzer" (Carlo Erba, ANA 1400, Carlo Erba, Milano, Italy) following the principle of the Dumas combustion process. The outlet of the nitrogen analyzer delivers the N sample as N_2 in a stream of He gas. This outlet was coupled via an open split device to a mass spectrometer, which was continuously analyzing the gas mixture. The mass spectrometer was a dual inlet isotope ratio mass spectrometer (VG, type SIRA-10, VG Isotech Div of Fisons Instruments, Middlewhich, U.K.). Samples were determined in duplicate, resulting in a precision of ± .001 ^{15}N atom percent excess (APE).

Data Analyses. Total and endogenous flow of N at the terminal ileum were expressed relative to DMI. These calculations together with the determination of the daily DM flow were based on the chromium ratio marker method as described by Furuya and Kaji (1992).

The amount of ileal endogenous N (grams/kilogram of DMI) was calculated from the ratio of ^{15}N enrichment excess in ileal digesta (E_d) to that in the blood plasma TCA-soluble fraction (E_b) using the adapted formula according to Souffrant et al. (1981) and de Lange et al. (1990):

$$\mathbf{N_e = N_d \times [(E_d - nE_{feed})/(E_b - nE_b)]} \qquad [1]$$

where N_e = the endogenous N flow; N_d = N in the ileal digesta (grams/kilogram of DMI); nE_{feed} = the natural ^{15}N enrichment of the experimental feed; and nE_b = the natural ^{15}N enrichment of the blood plasma.

For the calculation of the ileal endogenous N excretion, the measured ^{15}N excess in the samples of ileal digesta collected on d 7, 8, and 9 of the infusion period and the corresponding ^{15}N excess in the TCA-soluble blood plasma were used.

Statistical Analyses. The effect of dietary treatment and day of ileal digesta collection on DM and total and endogenous N flow at the terminal ileum were analyzed by GLM procedure of SAS (1990) according to the following model:

$$\mathbf{Y_{ijkl} = \mu + T_i + A_k(T_i) + D_j + (D_j \times T_i) + e_{ijkl}} \qquad [2]$$

where, Y_{ijkl} = dependent variable, μ = overall mean, T_i = treatment (i = 1, 2, and 1, 2, and 3

for Exp. 1 and 2, respectively), D_j = day of collection (j = 7, 8, and 9), A_k = animal, and e_{ijkl} = residual error. The effect of treatment (T) was tested against animals within the treatment [A(T)]. Day effect (D) and the effect of interaction of day and treatment (D x T) were tested against the residual error (e).

Results

Pigs remained healthy and consumed their daily allowances throughout the experiments. Due to diarrhoea of one pig of the NDF-free group on d 8 and 9 (Exp. 1), ileal chyme was collected only on d 7. Similarly from one pig of the WB treatment group (Exp. 2), ileal chyme was collected only on d 8 and 9. For the same reason, one pig of the SFH dietary treatment group was excluded completely. Complete ileal digesta was collected from all other pigs. A dissection after the experimental period showed that all pigs had no irregularities after cannulation and catheterization.

Results on the chemical composition of the experimental diets are given in Table 1. The results showed that the N content was similar for all diets. In the WB diet, some extra N was present because of N-containing substances in the wheat bran. Wheat bran was included at the expense of glucose and starch. Analyses by Schulze et al. (1994) detected an amount of 179 g of starch/kg of DM of wheat bran. In the present study, dietary fiber sources rich in hemicellulose, cellulose, and lignin as determined by NDF analyses were used. The NDF content of the fiber sources used in the present study was 910.9, 490.0, and 720.1 g of NDF/kg of DM for pNDF, WB, and SFH, respectively. Further characterization of the NDF of the various dietary fibers sources according to literature showed that the composition of the insoluble fibers, WB and pNDF, was similar. According to Schulze et al. (1994) the NDF of WB consisted of approximately 69% of hemicellulose, 25% of cellulose, and 6% of lignin. The insoluble fiber of pNDF consisted of 63% of hemicellulose, 27% of cellulose, and 10% of lignin. However, the NDF of SFH consisted of 27% of hemicellulose, 53% of cellulose, and 20% of lignin (CVB, 1992). Consequently, the SFH diet contained approximately twice as much cellulose and lignin; however, less than half the amount of hemicellulose than the WB and pNDF diet. The starch content in the WB was 179 g/kg of DM (Schulze et al., 1994). This demonstrates that the product contained an appreciable quantity of wheat endosperm. Graham et al. (1986) used wheat bran that contained 156 g of starch/kg of DM. They also measured that the soluble fraction of non-starch polysaccharides in the wheat bran was only 7%. Therefore, the fiber fraction in our diets can be considered as mainly insoluble.

Statistical analysis according to the repeated measurement model Equation [2] showed

that there were no collection day effects ($P > .05$) on parameters of ileal digesta with regard to daily DM flow and the total and endogenous N flows .

Experiment 1. The addition of 200 g of pNDF/kg of diet increased the daily flow of DM ($P < .001$) as well as the total ($P < .01$) and endogenous N flow ($P = .053$) at the terminal ileum (Table 2). The proportion of endogenous N in the total N excretion (g of N/kg of DMI) was similar for the NDF-free and the pNDF1 diet (70.6 and 66.8%, respectively; Table 2). The additional total N flow of 1.884 g/kg of DMI with the pNDF1 diet consisted of 59% of endogenous N (1.112 g/kg of DMI) and of 41% of exogenous N (.772 g/kg of DMI).

Experiment 2. The inclusion of pNDF, WB, and SFH in a basal semi-synthetic diet at equal dietary NDF levels did not affect the daily flow of DM (grams/day) and the amount of endogenous N (grams/kilogram of DMI) passing the terminal ileum ($P > .05$; Table 2). The amount of total N (grams/kilogram of DMI) and DM passing the terminal ileum, however, was influenced by the various dietary fiber sources ($P < .05$; Table 2). The amount of total N (grams/kilogram of DMI) from endogenous origin passing the terminal ileum were 79.4, 68.5, or 80.5% with feeding the pNDF2, WB, or SFH diets, respectively. With the WB diet, the total N flow was increased by 1.406 g of N/kg of DMI when compared with the pNDF2 diet. Of this increase, 35% was of endogenous origin (.499 g of N/kg of DMI) and 65% was of exogenous origin (.907 g of N/kg of DMI).

TABLE 2. Least square means of dry matter and nitrogen flows at the terminal ileum as affected by different dietary treatments of NDF

	Experiment 1				Experiment 2				
Item	NDF-free[a]	pNDF1[a]	SEM	P-value	pNDF2[a]	WB[a]	SFH[a]	SEM	P-value
Number of pigs	5	5			4	4	3		
Number of observations	13	15			12	11	9		
Ileal dry matter flow, g/day	45.9	173.5	2.80	< .001	140.2	151.0	144.2	5.33	.375
Ileal dry matter flow, g/kg of DMI	85.3	318.9	8.80	< .001	214.0	243.6	230.7	6.97	.040
Total ileal N flow, g/kg of DMI	3.879	5.763	.326	.004	4.026	5.432	4.201	.274	.014
Endogenous ileal N flow, g/kg of DMI	2.740	3.852	.347	.053	3.224	3.723	3.382	.222	.302

[a] NDF-free means basal experimental diet without adding NDF, pNDF1 means basal experimental diet supplemented with 200 g of purified NDF/kg of diet, pNDF2 means basal experimental diet supplemented with 155 g of purified NDF/kg of diet, WB means basal experimental diet supplemented with 300 g of wheat bran/kg of diet, and SFH means basal experimental diet supplemented with 198 g of sunflower hulls/kg of diet.

FIGURE 1. Scheme of the experimental procedure

	preliminary period										experimental period								
Day	1	...	8	9	10	11	...	19	20	21	1	2	3	....	6	7	8	9	10
	Adaptation			PVTC[a]		Recovery			Catheters[b]		^{15}N infusion / collection ileal digesta[c]								D[d]

[a] Ileal cannulation of a Post-Valve-T-Caecum cannula (van Leeuwen et al., 1991).
[b] Insertion of two blood catheters.
[c] Collection of ileal digesta was carried out on d 7, 8, and 9 of the experimental period.-20
[d] Dissection of the animals.

Discussion

The level and source of fibrous products in the diet induce various physiological responses (Low, 1989; Graham and Åman, 1991) that can affect ileal nutrient flow.

In the present experiment, we observed an increase in DM flow at the terminal ileum when NDF was included in the diet. This increase agrees with earlier reports in the literature (Fernandez and Jørgensen, 1986; Graham et al., 1986; Partridge et al., 1986; Sauer et al., 1991; and Schulze et al., 1994) and can therefore be seen as the result of a lower digestibility of non-starch polysaccharides. Sauer et al. (1991) found similar effects on ileal DM flow by replacing cornstarch with Alphafloc or straw. Newton et al. (1983) demonstrated a similar effect of wheat bran diets. The ileal DM flow seems to be more dependent on the level of dietary NDF than on the various dietary NDF sources. We calculated an overall regression of the dietary NDF content (grams/kilogram) upon daily ileal DM flow (grams/day) on data of the present experiment (Figure 2) using the REG procedure of SAS (1990). The slope and intercept, both, differed from zero ($P < .05$). From the findings of the present study, it can be concluded that there is a close relation between the dietary NDF content and the daily ileal DM flow. However, the kind of NDF (if purified) did not have a great impact.

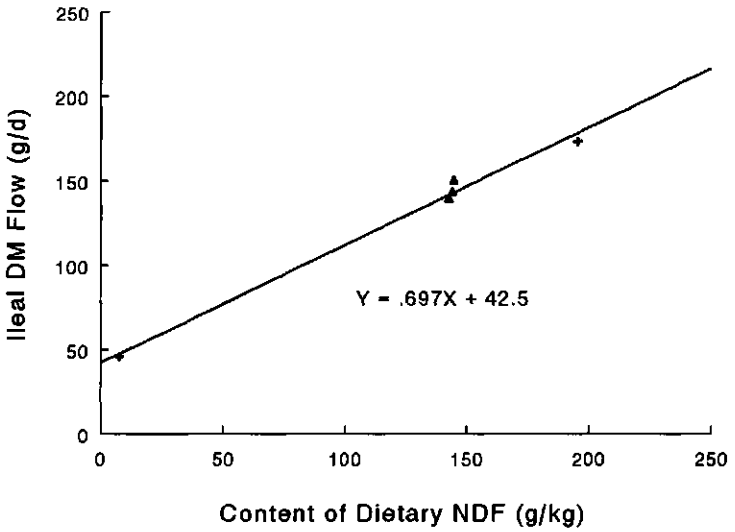

FIGURE 2. Effect of dietary content and source of neutral detergent fiber (g/kg of dry matter) on ileal dry matter flow (g/d) in pigs; $y = 42.416 + .697 \times X$ ($R^2 = .99$). Values are least square means for dietary treatment of Exp. 1 (+) and dietary treatments of Exp. 2 (▲). For details of diets, see Table 1.

The amount of total N passing the terminal ileum depends on both, the level and source of dietary NDF (Table 2). Effects of fibers on mechanisms involved in secretion and absorption of nitrogenous substances induce these changes (Low, 1989; Graham and Åman, 1991). It has been reported that endogenous N secretions including, pancreatic protein and juice (Langlois et al., 1987), bile (Portman et al., 1985), mucus (Low, 1989), and sloughed epithelial cells (Shah et al., 1982) are secreted in larger amounts when experimental animals are fed purified diets supplemented with fiber than in animals fed purified diets not supplemented with fiber. However, increased ileal N flow rates also can be affected by the rate of absorption (Low, 1985; Rerat, 1985; Vahouny and Cassidy, 1985). Some properties of the NDF that can influence absorption are: 1) a retained water-holding capacity during the passage through the gut (Dierick et al., 1989; Chesson, 1990), 2) the adsorption of amino acids and peptides on the fiber (Bergner et al., 1975; Mitaru et al., 1984), and 3) alteration of the transit time through the intestinal tract (Graham and Åman, 1991).

The quantity of total N passing the terminal ileum is dependent on both the remaining dietary N and on endogenous N that is not re-absorbed. It is important that both quantities are known (Huisman et al., 1993). However, in order to distinguish between the endogenous and exogenous N fraction, specific experimental and analytical methods are required. Although not entirely without weaknesses (De Lange et al., 1992; Moughan et al., 1992), the ^{15}N-isotope dilution method is one of the most used methods to determine the endogenous N flow at the distal ileum of pigs fed diets containing protein (i.e. De Lange et al., 1990; Souffrant et al., 1991, 1993; Huisman et al., 1992). Moreover, there is a good agreement between the ileal endogenous N flow estimated with the NDF-free diet and the ileal endogenous N according to Butts et al. (1993). Butts et al. (1993) derived an ileal endogenous N flow of 2.92 g/kg of DMI. This value is similar to the ileal endogenous N flow of 2.74 g/kg of NDF-free dry matter intake as given in Table 2 with the ^{15}N-isotope dilution method.

In the present study, the inclusion of 200 g/kg of dietary NDF increased the amount of endogenous N passing the terminal ileum compared to the NDF-free diet. These findings agree with those of Sauer et al. (1977) and Taverner et al. (1981). Other investigators (De Lange et al., 1989; Furuya and Kaji, 1992; Leterme et al., 1992) reported that additional dietary NDF increased the ileal endogenous N flow in pigs to a smaller extent (not significant). In the present study, the inclusion of 155 or 200 g of pNDF/kg of diet (142.8 or 195.5 g of NDF/kg of DM) caused an additional ileal endogenous N flow of .484 and 1.112 g of N/kg of DMI, respectively, compared with the NDF-free diet. Schulze et al. (1994) calculated after inclusion of 60, 120, and 180 g of pNDF/kg of diet (60.9, 120.8, and 176.5 g of NDF/kg of DM) an additional ileal endogenous N flow of .076, .559, and 1.128 g of N/kg of DMI, respectively. A regression of additional ileal endogenous N flow (grams/kilogram of DMI) on the dietary NDF content (grams/kilogram) with data of the present experiment using pNDF as fiber source and the data reported by Schulze et al. (1994) using the REG procedure of SAS (1990) was carried out. The slope and the intercept differed from zero with $P < .05$ and $P = .13$, respectively (Figure 3). The results show a linear increase of endogenous N up to a NDF level of 200 g/kg of DM. This contradicts the results of Taverner et al. (1981) somewhat, who observed no further increase in endogenous N above dietary NDF levels of 100 g/kg of DM.

The dietary inclusion of SFH caused similar ileal endogenous N flow compared with pNDF. When WB, however, was included in the diet, there was an additional endogenous ileal N flow of .499 g/kg of DMI compared with the pNDF2 diet. Because pNDF was isolated from this wheat bran, the soluble fibers removed during the isolation procedure may be responsible for this difference (Graham and Åman, 1991).

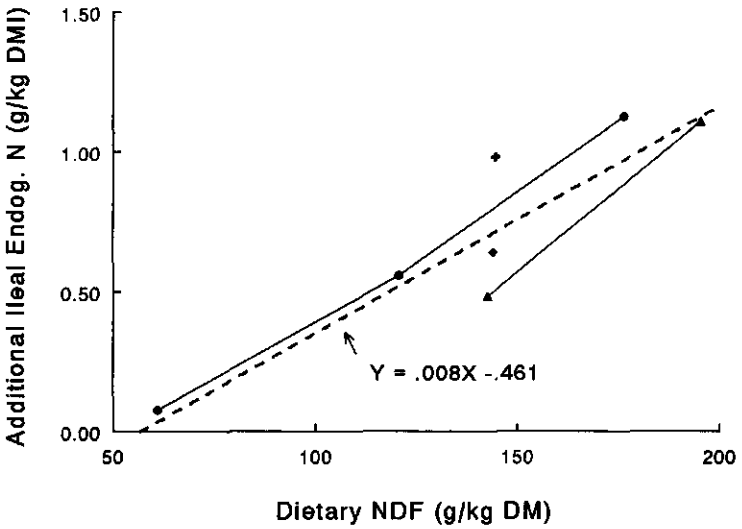

FIGURE 3. Effect of dietary content and source of neutral detergent fiber (g/kg of dry matter) on the additional ileal endogenous N flow (g/kg dry matter intake) in pigs;
Y = -.461 + .008 x X (R^2 = .93). Values are least square means for dietary inclusion of purified NDF in Exp. 1 and 2 (▲), for dietary inclusion of wheat bran in Exp. 2 (+), sunflower hulls in Exp. 2 (◆), and the means for dietary inclusion of purified NDF as used by Schulze et al. (1994) (●).

From the present study it can be concluded that ileal DM and N flow increase with inclusion of purified NDF in the diet. The increase in ileal N is both an increase in endogenous and exogenous N. When NDF is part of an dietary ingredient, the total increase in ileal N may be greater than from purified NDF.

Implication

The present study established that increases in dietary neutral detergent fiber content are associated with increases in the ileal dry matter flow. Results indicate, that neutral detergent fiber in both purified and complex form, will reduces N utilization in growing pigs as a result of increase of both endogenous and undigested dietary ileal N losses. This means that diets rich in neutral detergent fiber (i.e. 20%) may be supplemented with approximately 10 g ileal digestible protein/kg of diet to compensate these losses.

Further studies on the effects of natural fiber sources rich in insoluble fibers, combined with increasing amounts and various sources of soluble fibers, on the ileal endogenous and exogenous N flow are required.

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Chapter 5

Soybean Trypsin Inhibitors Affect Ileal Endogenous and Exogenous Nitrogen Flow in Pigs

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Soybean Trypsin Inhibitors Affect Ileal Endogenous and Exogenous Nitrogen Flow in Pigs

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Abstract: A study was conducted with 12 male castrated pigs (average BW 13 kg) to determine the effect of various levels of purified soybean trypsin inhibitors (sTI) in the diet on ileal nitrogen flow. To distinguish between endogenous and exogenous N passing the terminal ileum, the ^{15}N-isotope dilution method was used. Pigs were fitted with a post-valvular T caecum cannula and two indwelling blood catheters. They were fed 500 g/d of a corn starch-based semi-synthetic diet supplemented with 0, 2.4, or 7.2 g of sTI/kg of diet. The commercial purified sTI containing Kunitz and Bowman-Birk inhibitors was administered for 10 d. Trypsin inhibitor activity (TIA) of the three inhibitor supplemented test meals was .21, 2.49, and 5.77 g/kg of diet, respectively. The dietary inclusion of 2.4 or 7.2 g of sTI/kg of diet increased ($P < .05$) the daily flow of total N at the terminal ileum by 1.627 or 4.053 g/d, respectively, compared with the sTI-free diet. Endogenous ileal N was increased with 1.061 or 2.132 g/d when 2.4 or 7.2 g of sTI/kg of diet, respectively, were included. The addition of 2.4 or 7.2 g of sTI/kg of diet also increased the amount of undigested feed N at the terminal ileum by .566 or 1.921 g/d, respectively. The pancreatic weight from the pigs fed increasing amounts of added sTI were not different ($P > .05$). No alterations ($P > .05$) of trypsin and chymotrypsin activity in the pancreatic tissue were observed after feeding diets with increasing TIA.

Key Words: Pigs, Trypsin Inhibitor, Ileal Endogenous Nitrogen, ^{15}N-isotope Dilution Method, Proteolytic Enzymes, Pancreas

Introduction

The importance of soybean as a protein supplement for animal feed and its potential value for human food is well recognized. The principal drawback in the utilization of soybean protein, apart from the deficiency in sulfur-containing amino acids, is the presence of natural constituents that may adversely affect its nutritive value (Kakade et al., 1972).

The trypsin inhibitor proteins (TI) are one of the major antinutritional factors (ANF) in

soybean seed (Borchers et al., 1948; Huisman and Jansman, 1991). In soybean seed, TI consists of two major types, the Kunitz (Kunitz, 1947 a,b) and Bowman-Birk (Bowman, 1944; Birk, 1961) inhibitors. A systematic approach to quantify the effects of TI on the nutritional value of diets and the effect of these ANF on the passage of N to the terminal ileum can be the use of purified protease inhibitors (Le Guen, 1993). Barth et al. (1993) reported a dose dependent enhanced appearance of both endogenous and exogenous protein at the ileum after feeding a single semisynthetic casein diet supplemented with various amounts of purified Kunitz inhibitor to guinea pigs. With meals composed of near isogenetic lines of soybeans containing low- or high-trypsin inhibitor activity (TIA) Herkelman et al. (1992) found that TI decreased the apparent ileal digestibility of nitrogen (N).

The purpose of the present study was to investigate the effect of various dietary amounts of purified soybean trypsin inhibitors (sTI) added to a nearly TI-free diet on the endogenous and exogenous N flow passing the terminal ileum in young growing pigs. To distinguish between endogenous N and undigested dietary N flow at the terminal ileum, the ^{15}N-isotope dilution method was used.

Materials and Methods

General, Animals and Housing. Twelve crossbred (Dutch Landrace x Yorkshire) castrated male pigs with an age of approximately 5-wk and an average BW (± SE) of 8.5 kg (± .1 kg) obtained from a commercial breeding farm were used. The animals were individually housed in 1.2-m x 1.2-m transparent smooth-walled metabolism cages and allotted randomly to one of the three dietary treatment groups. The average BW of the animals at assignment, at the beginning of the experimental period, was 11.1 kg (± .2 kg). At the end of the study the average BW was 13.1 kg (± .2 kg). The environmental temperature was maintained in a range of 23 to 26°C and the relative humidity was kept at 50 to 70%. Ethics approval for this study was given by the TNO-Institute for Nutrition and Food Research and Wageningen Agricultural University Animal Ethics Committees.

Diets and Feeding. During the preliminary period, the pigs were fed a semisynthetic soya concentrate based diet containing 23.6 g of N/kg (Diet A; Table 1). Approximately 150 g of the diet was given at 0800 and 2000 during the period before the ileal surgery. During recovery from surgery, the pigs were fed increasing amounts of the diet until they consumed 400 g/d at d 6 after surgery. The diet was fed four times a day in similar amounts, at 0600, 1200, 1800 and 2400. During the entire experimental period, the animals were fed 500 g/d of the

experimental diets. The various experimental diets were formulated by adding a commercially produced purified soybean trypsin inhibitor (sTI; Sigma, Chemical Co., St. Louis, MO) to a basal diet (Table 1), at 0 (Diet A), 2.4 (Diet B) or 7.2 g of sTI/kg of diet (Diet C). Chromic oxide was included in the diets at 1 g/kg as an indigestible marker. The diets were mixed with water (1:2, wt/vol) immediately before feeding and fresh water was available ad libitum for 30 min after each meal.

TABLE 1. Ingredient composition (g/kg, air-dry weight) of the experimental diet

Ingredient	Composition
Soya concentrate	240
Maize starch	458
Soya bean oil	25
Dextrose	150
Cellulose	50
Vitamin-mineral premix[1]	10
Minerals[2]	60
L-Lysine	2
DL-Methionine	2.5
L-Tryptophan	.50
L-Threonine	1
Chromic oxide	1

[1] Contributed the following per kilogram of diet: 9,000 IU of retinol; 1,800 IU of cholecalciferol; 40 mg of α-tocopherol; 1.36 mg of menadione dimethyl-pyrimidinol bisulfate; 5 mg of riboflavin; 40 μg of cobalamine; 30 mg of niacin; 15 mg of d-pantothenic acid; 120 mg of choline chloride; 50 mg of ascorbic acid; 2 mg of thiamin; 3 mg of pyridoxine; .1 mg of d-(+)biotine; 1 mg of folic acid; .38 mg of K (KI); .525 mg of Co ($CoSO_4$); .06 mg of Se (Na_2SeO_3); 80 mg of Fe ($FeSO_4$); 254 mg of Cu ($CuSO_4$); 44 mg of Mn (MnO_2); 72.8 mg of Zn ($ZnSO_4$); 40 mg of tylosin.
[2] Contributed the following per kilogram of diet: 15 g of $CaCO_3$; 22.5 g of monocalciumphosphate; 5 g of NaCl; 16 g of $KHCO_3$; 15 g of MgO.

Experimental Procedure. Each dietary treatment group comprised four pigs fitted with a postvalve T-caecum (PVTC) cannula (van Leeuwen et al., 1991) and two indwelling catheters, one into the external jugular vein (for taking blood samples) and the other one into the carotid artery (for infusion of the ^{15}N-Leucine solution). Detailed description of the experimental procedure has been presented by Schulze et al. (1994). During the 10-d experimental period

a constant 10-d ^{15}N-Leucine infusion was performed at a rate of 5.04 mg of ^{15}N-Leucine (95% ^{15}N enrichment) kg^{-1} of BW d^{-1} according to the procedure described by Schulze et al. (1994). Ileal digesta was collected continuously for 12 h on d 7, 8, and 9 of the infusion period. The collected digesta were immediately frozen and stored at -20°C.

Blood samples were taken three times a day, at 0900, 1500, and 2100. The day before the infusion of the ^{15}N-Leucine solution, blood was taken to determine the background ^{15}N content of the trichloroacetic acid (TCA)-soluble fraction of the blood plasma. Samples of blood were prepared for analyses as described previously (Schulze et al., 1994). Immediately after sampling, the blood was centrifuged (10 min, 1,000 x g). The supernatant was pooled per day and stored at -20°C.

On d 10, 3 h after their morning feeding, the animals were anesthetized with O_2/N_2O and halothane. The abdomen was opened and the pancreas was rapidly excised, trimmed of excess fat and lymph nodes, and weighed. Subsequently the pancreatic tissue was frozen and stored at -20°C. Thereafter, the animals were killed.

Chemical Analyses. Before analysis, the samples of ileal digesta and pancreatic tissue were freeze-dried, ground (< 1 mm), and thoroughly mixed. The experimental diets and daily samples of ileal digesta were analyzed for N and DM following AOAC (1984) prodedures. Chromium was determined according to Bosch et al. (1989). The experimental diets were analyzed for trypsin inhibitor activity (TIA) according to Van Oort et al. (1989). Pancreatic tissue was analyzed for trypsin (TA) and chymotrypsin activity (CTA) according to Bergmeyer (1974), after activation of the zymogen. The ^{15}N enrichment in ileal digesta, feed, and the TCA soluble fraction of blood plasma was determined using a dual-inlet isotope ratio mass spectrometer (VG, type SIRA-10, VG Isotech Div of Fisons Instruments, Middlewhich, England) by procedures reviously described (Schulze et al., 1994). Before measuring the ^{15}N enrichment, duplicate samples were taken from each sample and further pretreated for analyses as described previously (Schulze et al., 1994).

Data Analyses. The daily DM, total and endogenous N flow at the terminal ileum were analyzed according to the chromium ratio marker method as described by Furuya and Kaji (1992).

The contribution of endogenous N (grams/day) to the total N passing the terminal ileum was calculated from the ratio of ^{15}N enrichment in ileal digesta to that in the blood plasma TCA-soluble fraction as described by Schulze et al. (1994). For the calculation of the daily ileal endogenous N excretion, the measured ^{15}N excess in the samples of ileal digesta collected on d 7, 8, and 9 of the infusion period and the corresponding ^{15}N excess in the TCA-soluble

blood plasma were used. Ileal undigested dietary (exogenous) N was calculated by substracting endogenous N from total N passing the terminal ileum.

Statistical Analyses. The effect of dietary treatment and day of ileal digesta collection on DM and total and endogenous N flow at the terminal ileum were analyzed by GLM procedures of SAS (1990) according to the following model:

$$Y_{ijkl} = \mu + T_i + A_k(T_i) + D_j + (D_j \times T_i) + e_{ijkl} \qquad [1]$$

where, Y_{ijkl} = dependent variable, μ = overall mean, T_i = treatment (i= 1, 2, and 3), D_j = day of collection (j= 7, 8, and 9), A_k = animal, and e_{ijkl} = residual error. The effect of treatment (T) was tested with animals within the treatment [A(T)] as error term. Day effect (D) and the effect of interaction of day and treatment (D x T) were tested against the residual error (e).

Data on pancreas weight, TA, and CTA in freeze-dried pancreatic tissue were subjected to a one-way analysis of variance according to the procedure of SAS (1990). The significance of difference between treatments was tested by the Student's *t*-test (Steel and Torrie, 1980).

Results

Pigs remained healthy and consumed their daily allowances of feed throughout the experimental period. The dissection after the experimental period showed that the animals had no irregularities after cannulation and catheterization.

The chemical analyses of the experimental diets are presented in Table 2. The addition of various amounts of sTI increased the dietary N content by less then .05%. The commercially available sTI used in this study contains both the Kunitz and Bowman-Birk type inhibitors according to Technical Service Sigma. The analyzed trypsin inhibitor activity (TIA) in diets A, B, and C were .21, 2.49, and 5.77 mg of trypsin inhibited/g, respectively. In raw soybean seed, total TIA is as high as 20 to 30 mg of trypsin inhibited/g of seed (Huisman and Jansman, 1991). The levels of TIA in the diets were chosen to represent TIA levels commonly found in adequately toasted (Diet B) or undertoasted (Diet C) soya. Analyses of the soya concentrate for total lectin content using an ELISA-method (Hendriks et al., 1987) showed that the basal protein source used in this study was lectin-free.

TABLE 2. Chemical composition of the experimental diets

Item	Soybean trypsin inhibitor; g/kg of diet		
	0 (Diet A)	2.4 (Diet B)	7.2 (Diet C)
Trypsin inhibitor activity; mg/g[a]	.210	2.490	5.770
Nitrogen; g/kg of DM	26.21	26.34	27.19
Dry Matter; g/kg	901.81	902.39	902.00

[a] Trypsin inhibitor activity (TIA) expressed in milligrams/gram of air-dried material.

There were no collection day effects ($P > .40$) on total, endogenous, and exogenous N flow, and DM flow (Table 3). These findings suggest that the 6-d adaptation period to the experimental diets was adequate to measure DM an N flow at the terminal ileum on d 7, 8, and 9 of the experimental period.

TABLE 3. Statistical analyses of the effects of dietary treatment (T), animal (A), and day of collection of ileal digesta (D), and interactions on daily DM and total and endogenous N flow at the terminal ileum

Item	T	A(T)	D	D x T	e
DF	2	9	2	4	18
Dry matter flow; g/d					
Mean Squares	2393.7	97.948	5.769	32.408	21.460
F probabilities	< .001	.003	.767	.241	
Total N flow; g/d					
Mean Squares	49.924	.779	.068	.219	.151
F probabilities	< .001	.002	.646	.259	
Endogenous N flow; g/d					
Mean Squares	13.639	.654	.029	.133	.089
F probabilities	< .001	< .001	.728	.247	
Exogenous N flow; g/d					
Mean Squares	11.692	.524	.009	.012	.010
F probabilities	< .001	< .001	.413	.336	

The incremental addition of sTI to the semisynthetic diet caused a dose dependent increase in N flow at the terminal ileum ($P < .001$; Table 4). This increase is related to a reduction of apparent prececal N digestion from 85.0% in the absence of sTI down to 52.5% after ingestion

of 7.2 g sTI/kg of diet. With increasing dietary levels of sTI, of 0, 2.4, and 7.2 g/kg diet, 85.9, 76.5, or 63.5% of the total N passing the terminal ileum was of endogenous origin, respectively. Compared with the sTI-free diet the addition of 2.4 g of sTI/kg of diet increased the total ileal N flow by 1.627 g/d (192%). This increase consisted of 65% endogenous N (1.061 g/d) and 35% exogenous N (.566 g/d). With 7.2 g of sTI/kg of diet the total N flow passing the terminal ileum was increased by 4.053 g/d (328%) compared with the sTI-free diet. Of this increase, 53% was of endogenous origin (2.132 g of N/d) and 47% was of exogenous origin (1.921 g of N/d). Dietary sTI did not affect ($P > .05$) pancreatic weight or TA and CTA in the pancreas (Table 5).

TABLE 4. Dry matter and N flow (g/d) at the terminal ileum as affected by incremental inclusion of purified soybean trypsin inhibitors[a]

	sTI[b]	0	2.4	7.2	Pooled	Level of
Item	TIA[c]	.210	2.49	5.77	SEM	significance
Dry matter flow		79.80[d]	93.12[e]	108.03[f]	2.86	***
Total N flow		1.777[d]	3.404[e]	5.830[f]	.255	***
Disappearance[g]		84.96[d]	71.36[e]	52.46[f]	2.10	***
Endogenous N flow		1.545[d]	2.606[e]	3.677[f]	.233	***
Exogenous N flow		.232[d]	.798[d]	2.153[e]	.209	***

[a] Data are means of four pigs averaged over three collection days.
[b] Soybean trypsin inhibitor added to the experimental diet in grams/kilogram of diet.
[c] Dietary trypsin inhibitor activity expressed as grams of trypsin inhibited per kilogram of diet.
[def] Means with different superscripts differ, $P < .001$.
[g] Disappearance of N expressed as a percentage of N intake.

TABLE 5. Pancreas weight and proteolytic enzyme activities as affected by incremental inclusion of purified soybean trypsin inhibitors[a]

	sTI[b]	0	2.4	7.2	Pooled	Level of
Item	TIA[c]	.210	2.49	5.77	SEM	significance
Total w; g/kg of BW		1.838	1.767	1.833	.164	NS
Trypsin activity[d]		3002	3029	1927	465	NS
Chymotrypsin activity[d]		866.0	817.5	471.0	193.4	NS

[a] Data are means of four pigs averaged over three collection days.
[b] Soybean trypsin inhibitor added to the experimental diet in grams/kilogram of diet.
[c] Dietary trypsin inhibitor activity expressed as grams of trypsin inhibited per kilogram of diet.
[d] Units per gram of air-dry matter.

Discussion

The mean values for apparent disappearance of N in soybean meal without adding sTI, determined at the terminal ileum agree with other reported values (Chang et al., 1987; Knabe et al., 1989; Herkelman et al., 1992).

The aim of the present study was to determine the effect of endogenous, exogenous, and total N flow at the terminal ileum in pigs fed diets varying in the amount of added sTI. For this purpose, the ^{15}N-isotope dilution method was used. This method allows quantitative differentiation of excreted undigested dietary and exogenous N after feeding protein-containing diets (Souffrant et al., 1981). Although the assumptions and validation of this technique are still subject of scientific discussion (De Lange et al., 1992; Moughan et al., 1992), with protein containing diets, determination of endogenous N with this method gave similar results as the homoarginine method (Hagemeister and Roos, 1991). The result on ileal endogenous N flow of 1.545 g/d for the sTI-free diet determined with the ^{15}N-isotope dilution method were also similar to that calculated from linear regression of daily ileal endogenous N flow on feed DM intake in a similar diet (Butts et al., 1993).

The findings of the present study are similar to those reported by Barth et al. (1993) wherein 3 g of Kunitz inhibitor (KI) was added to a single meal (approximately 13.3 g of KI/kg of diet), increased ileal endogenous N flow by 4.5 g. In our study, 7.2 g of sTI/kg of diet increased ileal endogenous N flow by 4.3 g/kg of ingested diet. Barth et al. (1993) concluded that the increase in ileal N flow after ingestion of protease inhibitors was more due to endogenous than to exogenous N. In relative terms in our study, the addition of 2.4 or 7.2 g of sTI/kg of diet increased the exogenous N flow 3.4- or 9.3-fold compared with a 1.7- or 2.4-fold increase in endogenous N flow, respectively. Furthermore, the additional exogenous N flow expressed per grams ingested of sTI/kilograms of diet was similar, .236 or .267 g/d by the addition of 2.4 or 7.2 g of sTI/kg of diet, respectively. The additional endogenous N flow, however, expressed per grams ingested of sTI/kilograms of diet was decreased from .442 to .296 g/d with the addition of 2.4 or 7.2 g of sTI/kg of diet, respectively. From these results, we concluded that with increasing amounts of sTI in the diet, exogenous N flow increased linearly. The reason for the differences between our findings and those of Barth et al. (1993) are possibly related to the duration of administration of isolated trypsin inhibitors. Barth et al. (1993) add KI to one meal and our experiment lasted 10 days.

Related to the ability of soya bean trypsin inhibitors to form stable, inactive complexes with the enzymes -trypsin and chymotrypsin- from the pancreas (Liener and Kakade, 1980) the observed increases of exogenous and endogenous N flow at the terminal ileum can possibly be explained by two mechanisms: 1) a decreased digestion and(or) 2) a reduced (re)absorption.

The inactivation of trypsin and chymotrypsin by trypsin inhibitors in the gut may reduce protein digestion. As long as the animal can produce and excrete sufficient proteolytic enzymes, the dietary protein is similarly digested and a decrease in ileal apparent digestibility of N is due to additional ileal endogenous N. Ileal flow of exogenous N and consequently the true ileal digestibility rate are thus not affected. However, in the situation where there is not enough trypsin and chymotrypsin activity for digestion of dietary protein, the ileal flow of exogenous N will be increased. That may have occurred with the ingestion of 2.4 or 7.2 g sTI/kg in the present study.

Feedback regulation, based on changed intra-luminal trypsin and chymotrypsin activity in the gut (Iwai et al., 1988; Fushiki and Iwai, 1989), can induce additional endogenous N secretion, which is due to an increased secretion of trypsin and chymotrypsin (Ozimek et al., 1985; Fukuoka et al., 1986). However, in contrast to rats (Grant, 1989), pancreas enlargement in pigs was not observed in our experiment. Findings with pigs by Yen et al. (1977) and Schulze et al. (1992) support the present results. An increase in pancreatic enzyme secretion could also result from an enhanced/intensified synthesis in the pancreas. This would result in changed enzymatic activity in pancreatic tissue. The inclusion of sTI in the diet did not change the pancreatic trypsin and chymotrypsin activity compared with the sTI-free diet. The inclusion of 7.2 g of sTI/kg of diet even tended to decrease pancreatic trypsin and chymotrypsin activity, which may suggest an exhaustion of the pancreas. These results agree with the results of other (Yen et al., 1977; Schulze et al., 1992; Le Guen,1993). These latter studies also showed that after feeding diets with high TIA to pigs, no increase in proteolytic enzyme activity in pancreatic tissue was observed. Furthermore, studies carried out on pigs fed diets with high or low TIA showed no effect on secretion of protease from the pancreas (Zebrowska et al., 1985; Corring et al., 1986; Buraczewska et al., 1991; Le Guen, 1993). Therefore, the net result of a negative feedback regulation on trypsin and chymotrypsin synthesis in relation to ingested trypsin inhibitors in pigs differs from that in humans and rats (Grant, 1989; Huisman, 1990).

Depending on the stability of the complexes formed between SBTI and pancreatic proteolytic enzymes, reabsorption of other endogenous nitrogenous sources may be altered. This means that the additional endogenous N may be influenced by level of reabsorption. In addition, the inhibition of protein digestion will increase amounts of N passing the small intestine. This may induce a stimulation of other endogenous secretions (Niess et al., 1972). The increasing DM flow with incremented dietary sTI can increase the amount of endogenous N passing the terminal ileum as reported by Van Bruchem et al. (1989) in sheep.

From the results of the present study, it can be concluded that the dietary content of protease inhibitors increase both endogenous and exogenous N flow at the terminal ileum in pigs. An increase in the amounts of ileal endogenous protein (N x 6.25) is associated with a

greater maintenance protein requirement. Together with catabolized amino acids, endogenous amino acid losses are the greatest contributor for maintenance amino acid requirement (Moughan, 1989). As a consequence, less dietary amino acids are available for lean growth.

Implications

The results of the present study indicate that soybean trypsin inhibitors reduce apparent ileal crude protein digestibility in pigs by increased flow rates of endogenous as well as exogenous crude protein. This means that trypsin inhibitor activity in the diet should be minimized to prevent reduced apparent protein digestibility.

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Chapter 6

Increased Nitrogen Secretion by Inclusion of Soya Lectin in the Diets of Pigs

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Increased Nitrogen Secretion by Inclusion of Soya Lectin in the Diets of Pigs

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Abstract: A study was conducted with twelve male castrated pigs of 13 kg average body weight (BW) to investigate the metabolic flow of purified soya lectin (SL) incorporated in pig diets in low and high doses and their effect on nitrogen (N) passing the terminal ileum. The pigs were fitted with a post-valvular T caecum cannula and two indwelling blood catheters. They were daily fed 500 g of a corn starch-based semi-synthetic diet, free or supplemented with low or high doses of purified lectin. To determine the proportion of endogenous N on the amount of total N passing the terminal ileum, the ^{15}N-isotope dilution method was used. The amount of dietary ingested total lectins, determined by an ELISA method, recovered in the stomach was reduced from 177 and 1,065 mg/kg of dry matter (DM) to 4.53 and 28.70 mg/kg of DM for the low lectin (LL) and high lectin (HL) diets, respectively. The concentration of lectins in mg/kg of DM in stomach and ileal digesta were at a similar level for LL and HL diets. The concentration of functional lectins as determined by the FLIA method (i.e. lectins capable of carbohydrate binding) were estimated in gastric digesta at 2.51 and 21.52 mg/kg of DM for LL and HL diets, respectively. They could not be detected in ileal digesta. The daily ileal DM and N flow was significantly increased ($P < 0.05$) when feeding the HL diet, as compared to the lectin-free (Control) and LL diets. The dietary inclusion of purified lectin increased ($P < 0.05$) the daily flow of endogenous N at the terminal ileum. With LL and HL containing diets, the ileal N flow were increased to 0.14 and 0.62 g day^{-1}, respectively, when compared to a Control diet. Endogenous N passing the terminal ileum was increased by 0.33 and 0.51 g day^{-1} for LL and HL diets, respectively. In addition the production of volatile fatty acids (VFA) which appeared in the ileal digesta, in particular acetate and proprionate was increased in the pigs that were fed with the lectin containing diets.

Key words: pigs, soya lectin, ileal endogenous nitrogen, ^{15}N-isotope dilution method, volatile fatty acids

at 0.1% (w/w) as an indigestible marker. The experimental diets were prepared and mixed with water (1:2, w/v) immediately prior to feeding. Fresh water was available for 30 min after each meal.

TABLE 1. Ingredient and chemical composition (g kg^{-1} air-dry weight) of the basal experimental diet.

Ingredient composition		Chemical composition	
Soya concentrate	240	Dry Matter; g/kg	901.8
Maize starch	458	Nitrogen; g/kg	23.64
Soya bean oil	25	TIA[3]; g/kg	.210
Dextrose	150	Lectins[4] (ELISA)	n.d.[6]
Cellulose	50	Lectins[5] (FLIA)	n.d.
Premix[1]	10		
Minerals[2]	60		
L-lysine	2		
DL-methionine	25		
L-tryptophane	.5		
L-threonine	1		
Chromic oxide	1		

[1] Contributed the following per kilogram of diet: 9,000 IU of retinol; 1,800 IU of cholecalciferol; 40 mg of α-tocopherol; 1.36 mg of menadione dimethyl-pyrimidinol bisulfate; 5 mg of riboflavin; 40 µg of cobalamine; 30 mg of niacin; 15 mg of pantothenic acid; 120 mg of choline chloride; 50 mg of ascorbic acid; 2 mg of thiamin; 3 mg of pyridoxine; .1 mg of d-(+)biotine; 1 mg of folic acid; .38 mg of K (KI); .525 mg of Co ($CoSO_4$); .06 mg of Se (Na_2SeO_3); 80 mg of Fe ($FeSO_4$); 254 mg of Cu ($CuSo_4$); 44 mg of Mn (MnO_2); 72.8 mg of Zn ($ZnSO_4$); 40 mg of tylosin.

[2] Contributed the following per kilogram of diet: 15 g of $CaCO_3$; 22.5 g of Monocalciumphosphate; 5 g of NaCl; 16 g of $KHCO_3$; 15 g of MgO.

[3] Trypsin inhibitor activity; in g inhibited trypsin/kg product.

[4] Total lectins determined using an ELISA-method; in g/kg product.

[5] Functional lectins determined using a functional lectin immuno assay (FLIA); in g/kg product.

[6] non detectable.

Ileal digesta were collected continuously on day 1, 3, 5, 6 and 7 of the experimental period for 6 hours per day in small plastic bags attached to the cannula and immediately frozen at -20°C. Prior to chemical analysis, the digesta samples were freeze-dried and finely (< 1 mm) ground. On day 7 of the experimental period two samples of 5 g of ileal digesta were collected per animal for the determination of volatile fatty acids (VFA). Immediately after collection the samples were acidified with 500 µl phosphoric acid (850 ml/l, reagent grade). Subsequently, the samples were frozen and stored at -20°C.

Blood samples (5 ml) were taken three times a day throughout the infusion period (09.00, 15.00, and 21.00 h). The day before the infusion of the ^{15}N-leucine solution, blood (10 ml) was taken to determine the background ^{15}N content of the trichloroacetic acid (TCA)-soluble fraction of the blood plasma. Immediately after sampling, the blood was centrifuged (10 min 1,000 x g). The plasma from each animal was pooled per day and stored at -20°C. The precipitate was discarded. Before chemical analysis for ^{15}N-enrichment, duplicate samples were taken from each sample. Forty percent (w/v) TCA (0.1 ml) was added to 0.5 ml of the pooled plasma samples and mixed. After overnight (16 h) storage at 4°C, this mixture was centrifuged at 3,000 x g for 20 min at 4°C. The precipitate was discarded, and the pH of the supernatant was adjusted to pH 7. Subsequently this mixture was freeze-dried.

On day 8 of the experimental period, three hours after their morning feeding, the animals were euthanized and dissected. The animals first received inhalation anasthesia with O_2/N_2O and halothane. The abdomen was opened and plastic strips were used to separate different segments of the gastro-intestinal tract. The stomach content and samples of three different places of the jejunum were taken, 0.5 m distal of the ligament of Treitz, the middle of the jejunum and 0.5 m proximal to the ileocaecal ligament, respectively. The digesta samples were frozen immediately and stored at -20°C. Prior to chemical analysis the stomach content for each animal was freeze-dried and finely (< 1 mm) ground.

Purification of soya lectins

Materials. Sepharose CL-4B was obtained from Pharmacia LKB Biotechnology Inc. (Uppsala, Sweden). Tween-20 was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). Bovine serum albumin (BSA), dithiothreitol (DTT) and N-acetyl-D-galactosamine were obtained from Sigma Chemical Co. (St. Louis, MO). 1,2-Phenylendiamin-dihydrochloride and glycerol was from Fluka. Microtiter plates (nr. 3590) were from Costar (Cambridge, MA). Divinylsulfon was from Merck. (Gal Nac-ß-O-CETE)$_n$BSA was obtained from Janssen, Biochimica. All other chemicals were of the best grade available.

Purification of soya lectins. Soya lectin (about 18 g) was extracted from defatted soya meal and purified in a two-step procedure including affinity chromatography on N-acetyl-D-galactosamine-Sepharose CL-4B and gel filtration on Sephadex G-25 using Pharmacia-LKB BioPilot equipment as will described by Hessing *et al.* (manuscript in preparation). Finally, the purified lectin preparations were lyophilized and frozen at -20°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) analysis of the isolated preparations under reducing conditions revealed the characteristic 30 kDa lectin subunits. IEF-analysis of the

purified lectin preparation revealed 2 major bands at IEP 5.2 and 5.65 and N-terminal sequencing analysis confirmed the identity of the purified soya lectin. Protein concentrations of the lyophilized lectins were determined using ELISA methodology (Hessing *et al* manuscript in preparation).

Chemical analysis

Enzyme-Linked Immunosorbent (ELISA) and Functional Lectin Immuno (FLIA) assays for the measurement of soya lectin. The basal diet, purified soya lectins, freeze-dried samples of ileal digesta and stomach content were evaluated for lectin content using both the ELISA and FLIA methods essentially according to Hamer *et al* 1989 for phaseolus vulgaris lectins, but modified for soya lectin (Hessing *et al*, manuscript in preparation). Briefly, either polyclonal anti-soya lectin antibodies (ELISA) or (Gal Nac-ß-O-CETE)$_n$BSA (FLIA) were coated to microtiter plates overnight at 4°C. Subsequently, the plates were blocked with 0.5% BSA and 0.2% Tween-20 in TBS for 1 h at 37°C. The plates were washed and reference soya lectin and samples were diluted at appropriate concentrations and transferred into the microtiter wells and incubated for 2 h at 37°C. The plates were washed and peroxidase-conjugated anti-lectin antibodies was applied and incubated for 2 h 37°C. Finally, the plates were washed and bound conjugated antibody was developed for peroxidase activity using 1,2-phenylenediamine. The absorbance at 492 nm was read and data were evaluated by the parallel line assay using a computer software package connected to the ELISA reader system (Eurogenetics, Belgium). All assays used the maximal number of data points on the linear part of the standard curve. Means and standard deviations were calculated with standard methods.

For the determination of lectin content, samples (1.0 g) of basal diet, ileal digesta and stomach content were suspended in 20 ml Tris-HCl buffer (50 mM, pH 8.2) and stirred vigorously on a magnetic stirrer for 1 h. Extracts were then centrifuged at 7,500 x g for 15 min and supernatants were used for serial dilutions and assay of lectin content. For the measurement of lectin content in ileal digesta, a serial dilution from 1/10 to 1/1280 was used and for stomach samples, a serial dilution ranging from 1/40 to 1/320 was prepared. Lectin contents were expressed as μg g^{-1} on dry matter basis.

Total nitrogen (N), dry matter (DM) and chromium were determined in the basal diet, freeze-dried samples of ileal digesta and stomach. The content of DM and N were determined according to AOAC (1984) procedures. Chromium was determined in the diet and in ileal digesta by the method of Bosch *et al* (1989).

For the determination of the endogenous N proportion of the total ileal excreted N analysis of ^{15}N enrichment was carried out in basal diet, samples of ileal digesta and the TCA-

soluble blood plasma. The ^{15}N-enrichments of total N in ileal digesta, diet and TCA-soluble plasma were measured using a dual inlet isotope ratio mass spectrometer (VG Isotech, Fison Instruments, Middlewhich, U.K.). Fifty ± 10 μg N of freeze-dried ileal digesta, TCA-soluble blood plasma and basal diet were placed into tin capsules (8 x 5 mm, Fr. Van Loenen Instruments). The tin capsules were combusted in a Total Nitrogen Analyser (Carlo Erba, ANA 1400, Fr. Carlo Erba, Milano, Italy) which was attached to the mass spectrometer. Samples were determined in duplicate.

Concentrations of VFA in ileal digesta were determined by a modification of the gas-liquid chromatographic method of Imoto and Namioka (1978) as described by Schutte *et al* 1992.

Data analysis

Ileal DM and N flows were determined three times in each pig, using the 6 h pooled digesta samples, collected on day 5, 6 and 7 of the experimental period. The daily flows of DM and N at stomach and terminal ileum were calculated using the content of chromium in the diet relative to chromium in ileal digesta (Furuya and Kaji, 1992). The daily flows of gastric and ileal total and functional lectins as well ileal VFA were estimated using the corrected DM flow.

Daily values for the ^{15}N-enrichment excess were subjected to non-linear regression (Proc NLIN, modified Gauss-Newton method, SAS 1990) according to the formula given by Souffrant *et al* (1993). From this it was derived that the ^{15}N-enrichment of total N in TCA-soluble blood plasma had reached a steady state after 6 day of continuous infusion, at ileal collection day 5, 6, and 7, respectively.

For the determination of endogenous loss using the ^{15}N-isotope dilution method, endogenous ileal N flows were calculated using the following equation:

$$N_e = N_d \times [(E_d - E_{nf})/(E_{pl} - E_{npl})] \quad [1]$$

where N_e is the endogenous N loss (g/day and g/kg of DMI); N_d is the total amount of N in the ileal digesta (g/day and g/kg of DMI); E_d is the ^{15}N-enrichment in ileal digesta; E_{nf} is the background ^{15}N-enrichment in the diet; E_{pl} is the ^{15}N-enrichment in the TCA-soluble blood plasma; and E_{npl} is the background ^{15}N-enrichment in the TCA-soluble blood plasma.

Statistical analysis

Parameters of total and functional lectin levels in stomach and ileal digesta and ileal VFA were subjected to a one-way analysis of variance according to the procedure of SAS (1990). The parameters were tested for their significance of difference using the Student's *t*-test (Steel and Torrie, 1980).

The effect of dietary treatment and day of ileal digesta collection on DM and total and endogenous N flow at the terminal ileum were analyzed by GLM procedure of SAS (1990) according to the following model:

$$Y_{ijkl} = \mu + T_i + A_k(T_i) + D_j + (D_j \times T_i) + e_{ijkl} \qquad [2]$$

where, Y_{ijkl} = dependent variable, μ = overall mean, T_i = dietary treatment (i= 1, 2, 3), D_j = day of collection (j= 1, 2, 3 for day 5, 6, 7, respectively), A_k = animal (k= 1,.. , 12), and e_{ijkl} = residual error. "A" represents the animal effect. The effect of treatment (T) was tested against animals (A). Day effect (D) and the effect of interaction of day and treatment (D x T) were tested against the residual error (e). The significance of the treatment on the parameter estimates was determined using the F-value which was calculated for the effect of treatment tested against animals. This method is similar to that reported by Schrama *et al* (1993) and is a powerful test to distinguish between treatment and time. Animals and residuals were assumed to be normally distributed.

RESULTS

Pigs remained healthy and consumed their daily allowances throughout the experiment. No difficulties were experienced in obtaining blood and ileal digesta samples from the pigs. The dissection after the experimental period revealed that the animals did not develop any irregularities upon cannulation and catheterization.

Ingredient and chemical composition of the basal diet (Control Diet) are given in Table 1. Lectin analyses of the basal diet and the included soya concentrate by ELISA and FLIA methods revealed that no lectins could be detected. The purified soybean lectins were analysed by the ELISA procedure using soybean lectin from Sigma as reference material and it was found that 63% of the amount of protein could be recovered as lectin immunoreactivity. The addition of 160 (Diet LL) and 960 mg SL (Diet HL) kg^{-1} of diet provided total and functional dietary lectin contents of 160 and 101 mg kg^{-1}, respectively, for Diet LL and 960 and 605 mg

kg^{-1}, respectively, for Diet HL.

Variable amounts of lectin levels were detected using ELISA and FLIA-techniques, indicating their survival in gastric and ileal digesta (Table 2). The concentration of total lectins (mg/kg of DM) at terminal ileum after 7 day exposure of the pigs to daily doses of 80 or 480 mg was almost the same as, or slightly less than that found in gastric digesta. Functional lectins (FLIA) could not be detected in the ileal digesta.

TABLE 2. Content of lectins (μg/g of dry matter) estimated in the ingested diet, gastric[1] and ileal digesta[2].

	Diet			SEM	Level of significance[3]
	Control	LL	HL		
Diet					
Total Lectin[4]	0	177	1,065	-	-
Functional Lectin[5]	0	112	671	-	-
Gastric digesta					
Total Lectin	n.d.[6]	4.53[a]	28.70[b]	1.23	***
Functional Lectin	n.d.	2.51[a]	21.52[b]	1.38	***
Ileal digesta					
Total Lectin	n.d.	9.67[a]	28.82[b]	2.72	**
Functional Lectin	n.d.	n.d.	n.d.	-	-

[1] Values are means for three animals of treatment each.
[2] Values are means for four animals of treatment each.
[3] *** $P < 0.001$; ** $P < 0.01$. Means with different superscripts are significantly different.
[4] Amount of total lectins determined using an ELISA-method.
[5] Amount of functional lectins determined using a functional lectin immuno assay (FLIA).
[6] non detectable

The calculated time-course of ^{15}N-enrichment excess of total N in TCA-soluble blood plasma and in ileal digesta is shown in Figures 1a-c. Data of various dietary treatment groups showed similar values of the ^{15}N-enrichment excess with time-course. Figures 1 a-c show that the calculated plateau values for TCA-soluble blood plasma ^{15}N-enrichment excess of 0.0300, 0.0263, and 0.0298 for the Control, LL and HL diets, respectively, were not reached. The estimated daily means however, were close to the curve and the calculated plateau value.

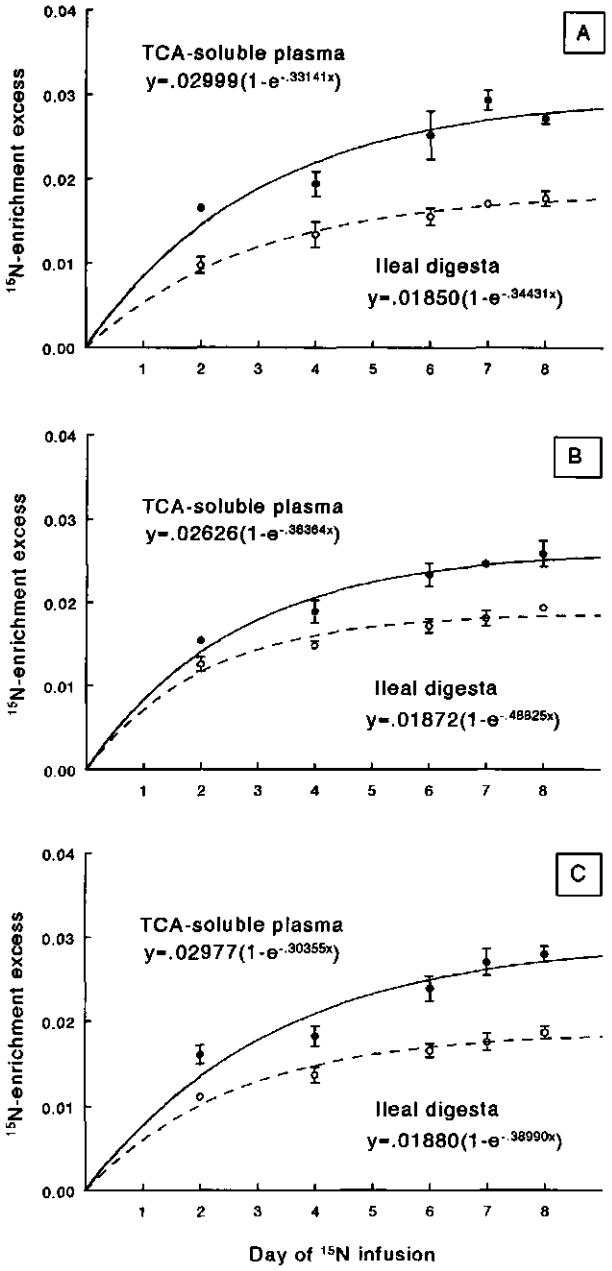

FIGURE 1. Overall time-course of ^{15}N-enrichment excess in total N of TCA-soluble blood plasma and ileal digesta, calculated on data of all animals within treatment (n = 4), when fed basal semi-synthetic diet supplemented with 0 (a), .160 (b), and .960 (c) g purified soybean lectins per kg of diet and continuously administered ^{15}N-leucine solution.

Statistical analysis according to the repeated measurement model (Equation 2) showed that there were no day effects ($P > 0.05$) on parameters of ileal digesta (Table 3) with regard to DM and total and endogenous N flows, using the data obtained on days 5, 6, and 7 of the experimental period. These data suggest that 4.5 d feeding of the experimental diets and 5.5 d continuous infusion of ^{15}N was adequate for major adaptation of animals and for measuring designed parameters at the terminal ileum on days 5, 6, and 7 of the experimental period.

TABLE 3. Results of the statistical analyses of the effects of dietary treatment (T), Animal (A), and collection day of ileal digesta (D) and interactions on daily dry matter (DM) and total and endogenous N flows at the terminal ileum and the dilution factor.

Item	T	A(T)	D	D x T	e
DF	2	9	2	4	17
Dry Matter flow; g/d					
Mean Squares	670.24	108.15	94.27	34.74	71.25
Level of significance[1]	*	NS	NS	NS	
Total Nitrogen flow; g/d					
Mean Squares	1.219	.137	.096	.049	.029
Level of significance	**	**	NS	NS	
Endogenous N flow; g/d					
Mean Squares	.723	.100	.024	.006	.024
Level of significance	*	**	NS	NS	

[1] ** $P < 0.01$; * $P < 0.05$.

The effects of various amounts of added purified soya lectins (SL) to a semi-synthetic basal diet on daily DM and total and endogenous N losses at the terminal ileum in pigs are presented in Table 4. The ileal DM flow of diet HL (960 mg of SL/kg of diet) was significantly increased ($P < 0.05$) as compared to the Control and LL (160 mg of SL/kg of diet) diets. The total ileal N losses of the various dietary treatments were significantly different ($P < 0.01$). Dietary inclusion of purified lectins significantly increased ($P < 0.05$) the endogenous ileal N losses compared to the SL-free (Control) diet. Endogenous N as a proportion of the total N flow at the terminal ileum were 62, 75, or 67% for the Control, LL, or HL diets, respectively. Compared to the SL-free diet, the addition of 160 mg of SL kg^{-1} increased the total ileal N flow by 0.144 g day^{-1} (8%). This additional total N flow was mainly caused by extra endogenous N (0.332 g day^{-1}) excreted. With 960 mg of SL kg^{-1} the total N flow passing the

terminal ileum was increased by 0.624 g day^{-1} (36%) compared to the SL-free diet. This increase accounts for 81% of N of endogenous origin (0.508 g of N day^{-1}).

TABLE 4. Means[1] of dry matter and N flows (g/day) at the terminal ileum of pigs fed a basal diet supplemented with 0 (Control Diet), 160 (Diet LL), or 960 (Diet HL) mg purified soybean lectins per kg of diet.

Item	Diet			SEM	Level of significance[2]
	Control	LL	HL		
Added lectins; mg/kg diet	0	160	960		
Dry Matter flow	80.52	84.37	95.30	3.08	*
Total N flow	1.757	1.901	2.381	.110	**
Endogenous N flow	1.088	1.420	1.596	.093	*

[1] Values are the means for four animals of treatment each. [2] ** $P < 0.01$; * $P < 0.05$.

Data presented in Table 5 showed that addition of SL tend to increase the bacterial activity as indicated by increased contents of VFA measured in ileal digesta. This increase, however, was not influenced by the ingested amount of purified lectins.

TABLE 5. Means of volatile fatty acids (VFA) in ileal digesta (mmol/day) of pigs fed a basal diet supplemented with 0 (Control Diet), 160 (Diet LL), or 960 (Diet HL) mg purified soybean lectins per kg of diet.

Volatile Fatty Acids	Diet			SEM	Level of significance[2]
	Control	LL	HL		
Added Lectins; mg/kg diet	0	160	960		
Acetic acid	4.6	9.1	9.6	1.7	.126
Proprionic acid	.6	1.9	2.2	.7	.260
Isovaleric acid	.5	.5	.6	.1	.432
Total VFA	5.8	12.2	12.4	2.6	.166

[1] Values are the means for four animals of treatment each.
[2] ** $P < 0.01$; * $P < 0.05$. Means with different superscripts are significantly different.

DISCUSSION

Information on the levels of lectins in soybeans and their relative toxicity in farm animals is not yet fully explained. Based on the haemagglutination activity (Valdebouze *et al* 1980), the lectin content of defatted soybean meal varies among cultivars from 1600 to 3000 units per mg (Gatel, 1992). However, in terms of total lectin activity estimates the haemagglutination test is inadequate (Huisman, 1989). Improvement in lectin detection and quantification have been made employing immunoassays often referred as ELISA and FLIA techniques (Hamer *et al* 1989). A lectin content between 10 and 20 mg g^{-1} meal as measured by the ELISA procedure is generally present in native soybean products (Huisman and Tolman, 1992). A lectin content of 4.5 and 0.05 mg g^{-1} meal in slightly and normally toasted soya flour has been reported by the latter authors. At the current lectin inclusion levels of 0.16 mg (Diet LL) and 0.96 mg (Diet HL) g^{-1} of diet, these figures represents approximately a normally toasted soybean meal and a meal containing approximately 10% raw soybean, respectively.

The effects of lectins on digestion and absorption of ingested nutrients is closely related to their own resistence to digestive processes and their presence on the luminal surface of epithelial cells (Pusztai *et al* 1990). In the present study, expressed as relative content, the amount of daily ingested total lectins which were found in the lumen of the stomach was about 3%, and at the terminal ileum about 1%. The lectin content was measured in the soluble fraction of the samples. This means that the lectin content of the non soluble fraction is not known. Lectin contents of the non soluble fraction, however, can hardly be measured. Thus the low lectin content in digesta of the stomach may reflect a way for lectin to pass the stomach without loosing their activity.

The lectin recovery of the present study is much lower than in the study of Pusztai *et al* 1990. They found, after 10 day of feeding a diet containing 0.7% purified soybean lectins in rats, about 60% in immunochemical intact form at the terminal small intestine. Thus suggesting that soybean lectins are highly resistant to small-intestinal proteolysis during the passage through the gastro-intestinal tract. The low concentrations of total and functional lectins found in pig ileal digesta in the present study may be caused by the extensively binding of N-acetylgalactosamine-specific lectins to the small intestine epithelium, as reported by Pusztai, 1991 and Pusztai *et al* 1990. Further, they are taken into enterocytes by endocytosis and appear to be released in the animal's system where a strong humoral antibody (IgG type) response against the lectin develops (De Aizupurua and Russell-Jones, 1988; Pusztai, 1991; Pusztai *et al* 1990). One of the consequences of the uptake of lectins by gut wall epithelium is a rapid increase in the cell protein synthesis in the mucosa (Oliveira *et al* 1988). Even a doubling of the small intestinal weight and an increase in gut protein synthesis of 70% was

found (Greer *et al* 1985; Palmer *et al* 1987).

Furthermore, lectin binding to the rat intestinal epithelium causes disruption of the brush border (Pusztai *et al* 1990), atrophy of the microvilli (Jindal *et al* 1984), and reduces the viability of the epithelial cells (Ishiguro *et al* 1992). According to Huisman and Jansman (1991) however, bindings of the lectins does not always cause damage to the brush border of the pig small intestine. Gut epithelial cell surface glycosylation patterns vary significantly with animal age and species (Alroy *et al* 1989; Taatjes and Roth, 1991). Therefore, the susceptibility of animals to dietary specific lectins may change with age and species (Grant and Driessche, 1993).

The total ileal nitrogen (N) losses (Table 4) for lectin-fed pigs were significantly higher ($P < 0.05$) than values calculated for the group on the lectin-free diet. Such increased losses of ileal N with adding purified soybean lectins to the diet can partially been explained by an intestinal mucus overproduction triggered by the presence of indigestible lectins (Freed and Buckley, 1978) and also by an impaired digestion and absorption of nutrients (Liener, 1986; Pusztai, 1989). Using the ^{15}N-isotope dilution method, which allows to distinguish undigested dietary and non-dietary ileal N, daily endogenous ileal N losses were determined. It was found that the daily amount of endogenous N passing the terminal ileum was increased with increasing amounts of dietary purified soybean lectins (Table 4). The rise of ileal N caused by ingestion of purified lectins was considerably more due to endogenous than to dietary N. For example, the increase of total N losses with an intake of 160 and 960 mg of SL/kg of diet were covered completely and for 81% by increased endogenous N loss, respectively. Hence, the influence of soybean lectins on metabolic amino acid economy is quantitatively more affected by a loss of endogenous protein (N x 6.25) than by nonabsorbed dietary protein. In the case of soya bean lectins it may be assumed that mainly increased mucus protein secretion caused this increase in endogenous N. The observed increased daily dry matter flow with the HL diet (960 mg of SL/kg of diet) compared to the LL (160 mg of SL/kg of diet) and lectin-free diets may be seen as a reduced digestion and absorption.

The observed increase in bacterial activity with added dietary purified lectins, as measured by the amount of volatile fatty acids in ileal digesta, are in accordance to the observation of Wilson *et al* 1980 and Banwell *et al* 1983. Grant and Driessche 1993 discussed a number of factors possibly causing the bacterial proliferation. Additional nutrient sources necessary for this additional bacterial growth were possibly provided by lectin-mediated mucus hypersecretion, epithelial cell loss, serum protein leakage and reduced digestion and absorption of dietary nutrients. According to Pusztai (1991) due to an increased epithelial cell turnover modulated by the lectins an increase in the number of potential binding sites for bacteria on the small intestinal epithelium supports the bacterial proliferation.

From the results of the present study it can be concluded that soybean lectins fed to pig cause increased ileal N losses. Since considerable amounts of the extra N losses are of endogenous origin, maintenance requirements of N are increased.

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General Discussion

GENERAL DISCUSSION

Introduction

The aim of livestock production is to supply the consumer with animal products of high quality at a reasonable price. Therefore, pig production systems aim to reach the animals' genetic potential for lean production. It is also important to obtain this body protein deposition with as little wastage of the ingested protein as possible (Moughan, 1991a). For several reasons losses of protein should be minimized. Firstly, these losses have consequences for dietary protein and energy requirements. Secondly, the excretion of nitrogen (N) contributes to environmental pollution. There are various physiological processes which influence the utilization of dietary N and influence the flow and avoidable losses of N in growing pigs. From Moughan (1993) the following groups of processes can be distinguished:

a) Digestion and absorption,

b) Use for body protein maintenance (integument, re-synthesis, gut) and

c) Inevitable and to over-capacity related amino acid catabolism.

These N losses could possibly be influenced if the mode of action of dietary factors on these processes were known. In this context, quantification of endogenous ileal protein losses and measurements of true ileal protein digestibility of dietary protein in pigs are essential. Endogenous protein (N x 6.25), is present in the digesta at the terminal ileum of the pig in significant quantities. To estimate the non-absorbed ileal dietary protein flow, corrections need to be made for the endogenous component. If the factors which influence endogenous ileal N losses are known, the following step is to remove those which increase these losses. This will reduce feed requirements and environmental N pollution.

The true digestible protein value is a fundamental property of the feed ingredient, regardless of the dietary conditions under which that ingredient is fed to the animal (Moughan, 1991a). The apparent digestibility, which comprises both true digestibility and endogenous N, is related to both dietary conditions and animal factors. Therefore, after removing the effect of variable endogenous excretion, true digestibility values will be more precise in detecting differences in digestibility caused by processing of the material (Moughan, 1991a). In addition, there is a trend to define amino acid requirements at the tissue level, using computerised simulation models and in this case the true coefficients of amino acid digestibility are more meaningful for dietary formulation (Moughan, 1991b).

Sources of endogenous ileal protein and amino acid losses in pigs.

Endogenous ileal loss is derived mainly from secretions of the gastrointestinal tract which comprise protein, peptides and amino acids from saliva, gastric, bile, pancreatic and intestinal secretions, plasma, and sloughed epithelial cells. Bacteria and ingested body hair are also included in the measurement of endogenous loss even though they are not strictly endogenous. In Table 1, are shown data on the amounts of endogenous N secreted and their relative proportion in the pig gastro-intestinal tract as summarized by Auclair (1986).

TABLE 1. The endogenous ileal nitrogen (N) secretion from various sources in pigs

Source of endogenous N secretion	Absolute N secretion g day^{-1}	Relative N secretion %
Saliva and gastric	2.0 - 3.3	9 - 11
Pancreas	2.5 - 6.7	11 - 23
Bile	1.8 - 3.0	8 - 10
Small intestine	14.4	65 - 49
Sloughed cells	1.4 - 2.0	6 - 7
Entire endogenous secretion	22.1 - 29.4	100

Not all of the secreted endogenous N reaches the terminal ileum of the pig. Part of the endogenous N secreted into the lumen of the gastro-intestinal tract is reabsorbed before the end of the ileum (73%, Krawielitzki et al., 1990; 79%, Souffrant et al., 1993). Consequently, net ileal losses of endogenous N, are the balance between the secretion and reabsorption of endogenous N. Therefore, increases in endogenous ileal N losses can be caused by: a) an increased secretion with no extra reabsorption, or b) a constant secretion with a decreased reabsorption (Schulze et al., 1993). The effects of various dietary factors on endogenous ileal losses are the consequences of the net results of secretion and reabsorption of endogenous N.

A variable but sometimes substantial but variable component of non-dietary ileal N losses are contributed by bacteria. The extent to which non-starch polysaccharides are degraded before they reach the caecum depends on microbial metabolism (Fuller, 1991). There are only a few data from literature concerning the bacterial ileal N content of pigs. These data show considerable variation, mainly caused by the ileal digesta collection method (Wünsche et al.,

1991), the analytical method (Rowan et al., 1992) and the diet fed to the pig (Drochner, 1984). According to Poppe et al. (1983), Drochner (1984) and Wünsche et al. (1991) bacterial ileal N proportion amounts to 25 - 30% of total ileal N. Dierick et al. (1983) calculated a content of 30 - 45% and Le Guen (1993) found that bacterial N proportion was about 30 - 50% of ileal digesta N. Our own results (Chapter 3) also showed more than 50% bacterial ileal N in pigs. Data on the amounts of ingested hair passing the terminal ileum are not available from the literature, although, losses of sulphur-containing amino acids by this source are worth considering.

Methods to determine endogenous ileal protein and amino acid losses in pigs.

A protein-free diet is still a commonly used method to determine endogenous protein losses in pigs. This, however, is a physiologically abnormal state (Low, 1980), which will probably influence body protein synthesis and prevent protein accretion (Millward et al., 1976; Muramatsu, 1990). It may also affect the amount of endogenous protein entering the gut. It has been clearly demonstrated (de Lange et al., 1990, Moughan and Rutherfurd, 1990; Moughan et al., 1992a; Butts et al., 1993a), that the traditional protein-free method leads to considerable underestimation of endogenous protein losses in simple-stomached animals. Further investigation of endogenous protein losses by the regression method (regressing ileal N on different amounts of diet or dietary protein and extrapolating), may also lead to errors. Taverner et al. (1981), Leibholz and Mollah (1988) and Mariscal-Landin et al. (1990), found similar or even lower values for endogenous amino acid and N losses by using the regression method, compared to those obtained after feeding pigs a protein-free diet. According to Souffrant (1991) it is not likely that a linear relation exists between feed intake and the amount of endogenous N loss. Changes in feed composition associated with variation in the protein level will also lead to changes in other crude nutrients and endogenous losses.
Alternative approaches to distinguish non-dietary protein from non-absorbed dietary protein at the terminal ileum in the pig under conditions of peptide and(or) protein alimentation are a) the homoarginine method (Hagemeister and Ebersdobler, 1985), b) the ^{15}N-isotope dilution method (Souffrant et al., 1981) and c) the recently developed peptide alimentation ultrafiltration method (Moughan et al., 1990).

The homoarginine method, a procedure to determine the endogenous ileal loss of lysine in simple-stomached animals, is based on a chemical transformation (guanidination reaction with *O*-methylisourea) of some of the lysine units in dietary protein to homoarginine (Hagemeister and Ebersdobler, 1985). Since homoarginine is not utilized for protein synthesis, quantitative indirect measurements of non-dietary protein at different levels of the precaecal

gastrointestinal tract are possible (Schmitz et al., 1991). It is assumed that homoarginine is released from the protein and absorbed to the same extent as lysine. If the conversion of dietary protein lysin to homoarginine is complete (Moughan and Rutherfurd, 1990), such an assumption is not needed. In addition, the direct determination of endogenous lysine losses from the gut are possible (Rutherfurd and Moughan, 1990). To estimate endogenous protein quantities (Schmitz et al., 1991; Barth et al., 1993), the absorption of homoarginine is considered similar to other dietary amino acids. When feeding guanidated protein for a longer period, the metabolism of the animal can be influenced due to an imbalance resulting from the absence of lysine, caused by the homoarginine (Tews and Harper, 1986).

An alternative and more general approach is the use of isotopes as marker substances. Used in the present studies and further commonly preferred, is the **^{15}N-isotope dilution method** (Souffrant et al., 1981). In this method, the animal's N pool is labelled by a continuous ^{15}N-leucine infusion. Assuming that the ^{15}N-enrichment of endogenous N is similar to that of the suitable endogenous N precursor pool, the contribution of endogenous N to total N in the ileal chyme can be calculated from the ratio of ^{15}N-enrichment in total N in ileal digesta and in the precursor pool. Central to this method is the choice of a suitable precursor pool for the endogenous N-containing material. The total N trichloroacetic acid (TCA)-soluble fraction of blood plasma is commonly used as the precursor pool. Another prerequisite is that for the isotope dilution method, a steady state is obtained for the level of marking the precursor N pool. A steady state condition is reached between 5 and 8 days of continuous infusion depending on the method and the amount of daily infused ^{15}N-leucine (Chapter 1, 2, 4, 5, and 6). Although the assumptions (mainly the ^{15}N enrichment of the TCA-soluble plasma N as the use of it as precursor N pool) and validation of this technique are still the subject of scientific discussions (de Lange et al., 1992; Moughan et al., 1992b), the ^{15}N-isotope dilution method is considered a reliable method to determine the endogenous N (Chapter 2) at the distal ileum of pigs independent of the dietary protein source. It is one of the most frequently used methods to determine endogenous protein (N x 6.25) losses in pigs (i.e. de Lange et al., 1990, 1992; Huisman et al., 1992; Mosenthin et al., 1993; Schulze et al., 1994). A potential source of error with this method may be the synthesis of intestinal mucosa from luminal dietary amino acids and subsequent secretions which are not enriched with ^{15}N (de Lange et al., 1992).

In contrast to the labelling of the animal's N pool the uniform labelling of the feed protein with ^{15}N also offers the possibility to determine directly the endogenous ileal amino acid and protein losses in pigs. A recently proposed method to produce uniformly ^{15}N-labelled soybean seeds by Grusak and Pezeshgi (1994) may lead to an increased application of this method in studying endogenous protein in humans and animals. Measurements of endogenous ileal N or amino acid losses by this method can, however, only be carried out for a short time

after administration of the labelled feed protein because the uptake of dietary amino acids and their incorporation into secreted proteins can be very rapid. Leterme et al. (1993) found 3 hours after feeding a ^{15}N-labelled diet an already high ^{15}N-labelling of the pancreatic juice.

The peptide alimentation ultrafiltration method (Moughan et al., 1990) is a new an relativly simple method to directly determine quantitative endogenous ileal amino acid and protein losses. The animal is fed a semi-synthetic diet containing enzymically hydrolysed casein (EHC; mol wt < 5,000 Da) as the sole N source. Ileal digesta are collected from the animal, and the endogenous protein (mol wt > 10,000 Da) is separated by centrifugation and ultrafiltration (Moughan and Rutherfurd 1990; Butts et al., 1993a). A limitation of this approach is that any endogenous peptides, free amino acids, urea and ammonium present in the digesta are also removed in the low-molecular-weight ultrafiltrate fraction, thereby underestimating the actual endogenous amino acid flows (about 11-21% of total digesta N, Moughan et al., 1990; Moughan and Schuttert, 1991; Butts et al., 1992; Butts et al., 1993a). This method can only be applied if the undigested protein residues of centrifugation and ultrafiltration, consist mainly of non-dietary protein.

Another approach to estimate endogenous amino acid losses, recently proposed by Butts et al. (1993a), is to feed the animal natural protein devoid of specific amino acids, and to measure the flows of those amino acids at the terminal ileum. One such protein, used by Butts et al. (1993a), is zein which is present in maize, and contains only traces of lysine and tryptophan. The endogenous ileal lysine losses found for the zein fed pigs and those using the peptide alimentation ultrafiltration method were not significantly different (Butts et al., 1993a). The limitations of this method are caused by the restriction of endogenous amino acid measurements on the devoided specific amino acid in the dietary protein, and that feeding an amino acid imbalanced diet for a longer period influences whole body protein synthesis. Further, the application of this approach is limited to a small number of protein sources.

Because of individual advances and limitations, the application of one of those alternative methods has to be closely related to the objectives of the study planned. It appears that the choice of method for determining endogenous amino acid and(or) N losses may strongly influence the resulting true digestibility coefficients for feedstuffs given to the growing pig (Butts et al., 1993a). Endogenous ileal protein (N x 6.25) losses in pigs estimated using various of these alternative (feeding protein/peptide containing diets) methods showed comparable results (Moughan and Rutherfurd 1990; Hagemeister and Roos, 1991; Butts et al., 1993a; Schulze et al., 1994). The objective of the present study was to investigate the effect of some dietary factors on the amount of endogenous protein passing the terminal ileum in pigs by using the ^{15}N-isotope dilution method. The first chapter focussed on the application of the ^{15}N-isotope dilution method for determining endogenous ileal protein losses in pigs. Firstly, we

tested whether the level of ^{15}N-leucine infusion influenced the estimated endogenous ileal protein. Daily infusion levels of a) 3 mg ^{15}N-leucine kg^{-1} liveweight and b) 30 mg ^{15}N-leucine kg^{-1} liveweight give the same results. It was concluded that endogenous N loss measurement is independent of the ^{15}N-leucine dose continuously infused within the range of 3 - 30 mg kg^{-1} liveweight day^{-1}. This allowed comparison of data obtained with different levels of ^{15}N-leucine infusion (Chapter 1). To determine endogenous ileal N losses with the ^{15}N-isotope dilution method as presented in Chapter 2, 4, 5, and 6 daily ^{15}N-leucine infusion rates of 5.04 mg kg^{-1} liveweight were used. In addition we also tested if the enrichment excess of various body N-pools with ^{15}N was similar (Table 2).

TABLE 2. The mean ^{15}N-enrichment excess in various body N-pools and in the ileal digesta N-pool in pigs continuously infused with ^{15}N-leucine.

Diet	Body N-pool			Ileal digesta N-pool
	TCA-soluble blood plasma	Small intestine	Pancreatic tissue	
SMP[1]	0.25	0.21	0.21	0.13
SBM[2]	0.23	0.22	0.21	0.14
SI[3]	0.24	0.22	0.23	0.22
FM[4]	0.23	0.24	0.26	0.15

[1] Skim milk powder, [2] Soybean meal, [3] Soya isolate, [4] Fish meal.

There were only small differences in ^{15}N-enrichment excess between the various body N-pools. This is an important observation because it shows that the TCA-soluble blood plasma N is a valid pool for the determination of endogenous ileal N using the ^{15}N-isotope dilution method. Further, it can be concluded that variation in the estimated endogenous N loss is caused mainly by the diet-dependent ^{15}N-enrichment of the ileal digesta N, and not by the body N-pool (Chapter 1). We also compared endogenous ileal protein losses determined using the ^{15}N-isotope dilution and the peptide alimentation ultrafiltration methods. Despite the basic differences in approaches as already mentioned, we obtained similar results for endogenous ileal N losses with these two methods (Chapter 2). This can be regarded as an indication that the ^{15}N-isotope dilution method may be used. In any way it gives confidence in the ^{15}N-isotope dilution method.

Dietary causes of variation in endogenous ileal protein losses

Different values for apparent ileal protein digestibilities are found following feeding of various diets to pigs. The observed variations in ileal protein losses are related to both a potential true digestibility of the ingested protein source, and animal and dietary factors which influence the digestion and absorption of dietary and non-dietary (endogenous) proteins in the gastro-intestinal tract of the simple-stomached animal. Subsequent studies aimed to investigate the effect of various dietary factors on endogenous ileal N losses in pigs.

The extent of endogenous ileal protein (N x 6.25) losses depends on various factors, such as:

- the intake of protein or peptides (Moughan and Rutherfurd, 1990; Butts et al., 1993a),
- the amount of protein intake (Krawielitzki et al., 1977; Butts et al., 1993b),
- the quality and structure of the protein (Gebhardt et al., 1981; Sauer and Ozimek, 1986),
- the protein status of the animal (de Lange et al., 1989b) and
- the presence of dietary protein in the digestive tract (Sauer and de Lange, 1992).

There are also effects of components other than protein in the feedstuff, and the pigs' diet which influence secretion, hydrolysation and/or reabsorption of endogenous protein (N X 6.25) in the gastro-intestinal tract of the simple-stomached animal. As reported in the literature these are:

- the level and composition of crude fibre (Sauer and Ozimek, 1986),
- the content and source of antinutritional factors (Huisman, 1990; Jansman, 1993),
- the dry matter intake (Butts et al., 1993b) and
- the passage of non-protein dry matter through the gastro-intestinal tract (van Bruchem et al., 1989).

In the present study, neutral detergent fibre (NDF) isolated from wheat bran, isolated trypsin inhibitors (TI) and lectins (LE) from soya, each at different levels, were used to investigate their effects on endogenous ileal protein losses in pigs using the ^{15}N-isotope dilution method. Different levels of NDF inclusion were used, as well as NDF of different sources. The use of isolated dietary factors was chosen to guarantee as far as possible, a mono-factorial approach. In this approach, interactions with other factors are not taken into account. In the literature, as already mentioned, endogenous N losses are considered to be related to the dry matter intake. Therefore, the experimental diets used in this study were balanced for equal dry matter intake. The decision to study the dietary effects on endogenous ileal protein losses in young growing pigs at about 35 days of age and a liveweight of about 12 kg during the experimental period,

was based on their relatively high sensitivity to changes in the diet administered, and the resulting physiological changes of the digestive system of the pig. The physiological changes related to weaning the pig, are extensively discussed by Makkink (1993).

The effect of **level and source of dietary NDF** on endogenous ileal N loss are reported in Chapters 3 and 4. The inclusion of water-insoluble fiber (NDF) in the diet induced an increase in the endogenous ileal N loss. By increasing the content of dietary NDF, additional endogenous N passed the terminal ileum of the pig. The increase in additionally excreted endogenous ileal protein, was found to be linear with increasing amounts of purified NDF from wheat bran. It was found that increasing amounts of dietary NDF also increased the ileal losses of undigested dietary protein. This increase could be explained by undigested dietary NDF bound N in ileal digesta. Although only a few levels were tested the increase seemd to be linear.

Results of the present study were not confirm with those after feeding graded levels of cellulose in a protein-free diet (Sauer et al., 1977; Green et al.; 1987; de Lange et al., 1989a; Furuya and Kaji, 1992; Leterme et al., 1992). The effects of various amounts of dietary purified NDF on endogenous ileal N losses in pigs may have been caused by the fibrous structure and the inclusion of hemicellulose, cellulose, and lignins in the NDF substrate used in our investigations.

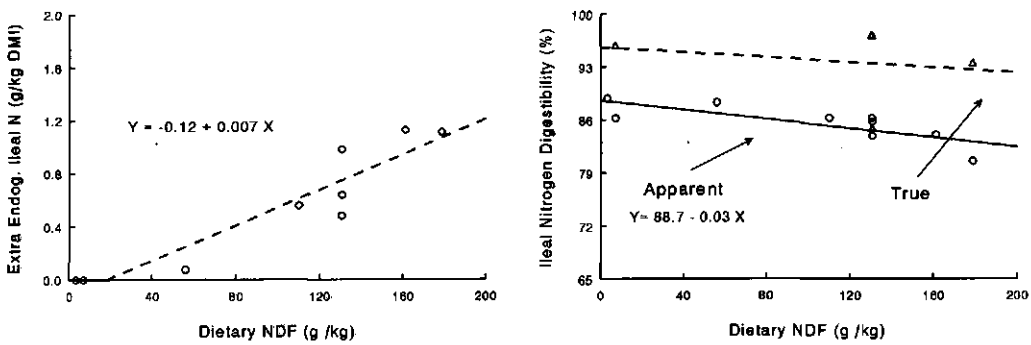
To investigate the relation between the source of dietary NDF and endogenous ileal N excretion, different fibre sources were included in a diet at similar dietary NDF level (Chapter 4). This study was done to assess endogenous ileal N losses related to the dietary NDF content. It was found that purified NDF from wheat bran and sun flower hulls with the same amount of NDF, induced similar amounts of endogenous ileal N losses. The losses with whole wheat bran, however, were higher than those observed with the other NDF sources including purified wheat bran NDF. The extra increase with wheat bran may be associated with other factors in bran which were removed by the isolation technique of the purified NDF. Probably, the effects of whole dietary fiber on endogenous ileal N losses will be greater due to other factors like viscous fibres (Larsen et al., 1993). Some properties of the fiber that can influence absorption are: a) a retained water holding capacity of pectins, mucilages and to a limited extent, hemicellulose during the passage through the gut (Schneeman, 1986; Dierick et al., 1989; Chesson, 1990), b) the adsorption of amino acids and peptides on the fiber (Bergner et al., 1975; Mitaru et al., 1984), and c) alteration of the transit time through the intestinal tract (Graham and Åman, 1991). It is still a matter of discussion as to which fiber properties increase endogenous N secretion. Taverner et al. (1981) assumed that mechanical stimulation of the mucosa by increasing the dietary fiber content increased mucin secretion. Observations

by Vahouny et al. (1985) support this assumption.

Consequently, the effect of dietary NDF on endogenous ileal N loss in pigs found in the present study, can be the result of increased secretion or decreased re-absorption of endogenous protein, or both. In our study we replaced dietary glucose with NDF. It could be questioned whether these effects of NDF on the endogenous ileal N loss might also be attributable to the removal of glucose from the diet.

Calculated from the data reported in Chapter 3 and 4, Figure 1 shows the effect of dietary NDF on extra endogenous ileal N loss. According to the equation given in Figure 1, it means that a diet containing 100 g NDF kg^{-1} may have an additional endogenous N loss, equivalent to at least 3.6 g protein kg^{-1} DMI.

The influence of dietary NDF content on apparent and true ileal N digestibility in pigs is given in Figure 2. As dietary NDF caused an increase in endogenous N losses, the major effect on ileal digestibility was expressed in apparent digestibility. The close relation of the apparent ileal N digestibility to the dietary NDF content could be given as an equation, which is included in Figure 2. There is also a tendency of a reduced true ileal N digestibility with increasing dietary NDF contents. It may be that the fibrous structure enclosing N which thus prevents digestion, may be a partial cause of the reduced true N digestibilities.



FIGURES 1 and 2. The effect of dietary NDF level (g kg^{-1}) on extra endogenous ileal nitrogen loss (g kg^{-1} dry matter intake) and on apparent and true ileal nitrogen digestibility (%) in pigs, respectively.

Anti-nutritive factors (ANF) are described as non-fibrous natural substances which can cause negative effects on growth or health in animals and humans. Mycotoxins and factors originating from processing, which can also have anti-nutritional effects, are excluded from this definition (Yannai, 1980). In plants and seeds, ANF act as biopesticides and give protection against moulds, bacteria, insects and birds (i.e. Liener and Kakade, 1980; Birk, 1987). These positive effects for the plant can cause serious disturbances in the animal and human after ingestion. This includes digestion, absorption and immunological reactions. In peas, common beans and soybeans, trypsin inhibitors and lectins are present at such levels that they may be classified as important (Huisman and Jansman, 1991). Therefore, studies presented in Chapter 4 and 5 of this thesis, were focused on the effect of trypsin inhibitors and lectins on endogenous protein passing the terminal ileum in pigs.

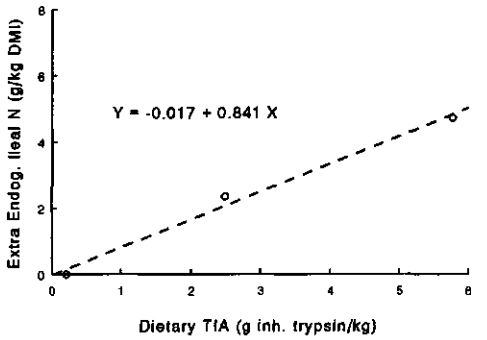
Among the protease inhibitors, the **trypsin inhibitors** are the most important for animal nutrition. Two types of trypsin inhibitors exist; the Bowman-Birk and Kunitz inhibitors, which differ in their molecular weight and disulfide bonds. These peptides can form stable, inactive complexes with the proteolytic enzymes -trypsin and chymotrypsin- from the pancreas (Liener and Kakade, 1980). By negative feedback mechanisms, the pancreas will produce more proteolytic enzymes to compensate for reduced enzymatic activity as induced by the complex formation. Pancreatic hypertrophy resulting from disturbed negative feedback induced by administered trypsin inhibitors has been shown to occur in small animal species (rat, mice and chicken) but not in larger farm animals such as pigs (Huisman and Jansman, 1991; Le Guen et al., 1991; Schulze et al., 1992, 1993). Furthermore, the complex of trypsin inhibitor with trypsin and/or chymotrypsin, may lead to an important loss of sulphur-containing amino acids, because these enzymes are rich in cystine and leave the small intestine (Liener and Kakade, 1980). Huisman and Jansman (1991) indicated that the effect of lowered apparent ileal protein digestibility with dietary trypsin inhibitors is mainly caused by an increased loss of endogenous protein.

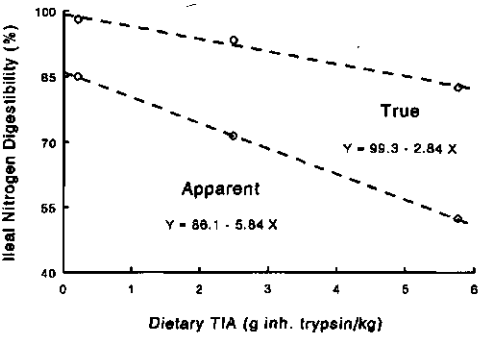
Various levels of isolated soybean trypsin inhibitors (sTI) were used in own studies reported in Chapter 5. It was found that trypsin inhibitors in the diet increased the endogenous ileal protein loss. Moreover, the endogenous ileal protein losses were positively related to dietary trypsin inhibitor levels. The relation between extra endogenous ileal protein loss and dietary content of trypsin inhibitors, measured as trypsin inhibitor activity, is shown in Figure 2.

The amount of endogenous ileal protein loss is the net result of both the secretion and reabsorption of endogenous protein. The reabsorption of endogenous protein may be influenced by the ability of the trypsin inhibitor to form stable complexes with trypsin and chymotrypsin, as mentioned before. According to Corring et al. (1986) no changes in total protein output from

the pancreatic juice occurred when raw soybeans were compared with heated soybeans. It may be questioned, however, if this latter observation is an effect of trypsin inhibitors present in raw soybeans. According to Le Guen (1993), changes in pancreatic enzymatic secretion also depend on true ileal protein digestibility of the feedstuff. Moreover, it was concluded from the findings of Corring et al. (1986), if there is an effect of dietary trypsin inhibitors on the secretion of endogenous protein, other sources of endogenous protein secretion should be increased. It seems, therefore most realistic that the increased excretion of endogenous N is most likely due to an decreased reabsorption of secreted pancreatic proteolytic enzymes. According to the equation given in Figure 3 it would mean that a diet containing 5 g trypsin inhibitor kg^{-1} diet would have an additional endogenous protein loss equivalent to at least 26 g protein kg^{-1} DMI.

The dietary trypsin inhibitor content also increased the ileal excretion of dietary protein (Chapter 4). To illustrate this, the effect of dietary trypsin inhibitors on apparent and true ileal protein digestibilities are given in Figure 4. The data showed a clear negative linear relationship between true and apparent ileal protein digestibility, and the dietary level of trypsin inhibitors in pigs.





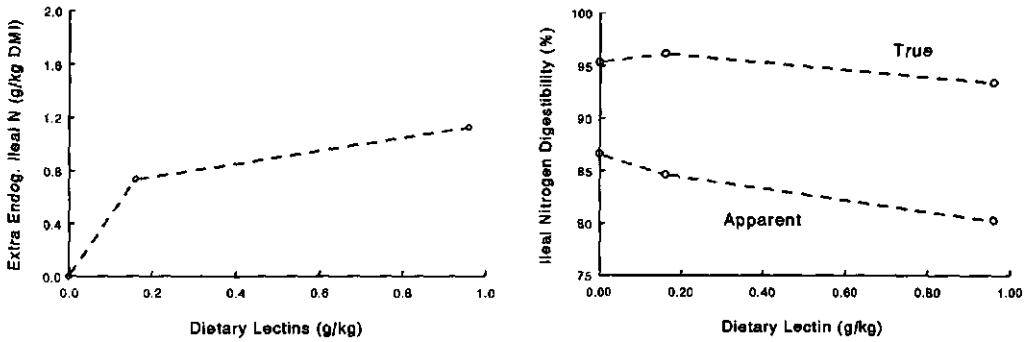
FIGURES 3 and 4. The effect of dietary trypsin inhibitor level (TIA = g inhibited trypsin kg^{-1}) on extra endogenous ileal nitrogen loss (g kg^{-1} dry matter intake) and on apparent and true ileal nitrogen digestibility (%) in pigs, respectively.

Isolated lectins from soybean were used to investigate the effect of dietarily administered **lectins** on endogenous ileal protein loss in pigs. Compared to phaseolus vulgaris lectins, soybean lectins are less harmful. These soybean lectins were chosen because soybean is the most used protein source in pig feed and relatively small effects can have important consequences in absolute terms.

Most lectins are able to bind to carbohydrate chains of glycoproteins and glycolipids (Köttgen, 1977). Both the membrane bound glycoconjugates of the glycocalyx (Egberts et al., 1984) and the free glycoconjugates in the mucus (Mouwen et al., 1983), are considered targets for these forms of binding. These bindings can be associated with a precipitation of glycoconjugates of the mucus, and a damaging effect on the intestinal epithelium (Greer, 1983; Köttgen, 1977). Related to the binding of lectins to the mucus in the gut wall epithelium, the cell protein synthesis in the mucosa increased rapidly (Olivera et al., 1988). This can lead to a doubling of the weight of the small intestinal wall (Palmer et al., 1987). Recent studies (i.e. Aletor, 1987; Aletor and Fetuga, 1988), with different legumes, have shown that ingested lectins depressed growth in the animals (mostly rats), by interfering with the digestion and absorption of nutrients in the gastro-intestinal tract. Decreased protein digestibility can be explained by increased mucin secretion as well as by greater numbers of bacteria (King et al., 1983; Greer and Pusztai, 1985).

Extra endogenous ileal protein losses were found after feeding isolated soybean lectins to pigs (Figure 5). Even a very small amount of ingested lectins, 160 mg kg^{-1} DMI, similar to well toasted soybean, caused an increase in endogenous ileal N loss compared to a lectin-free diet. A further increase of the dietary lectin level led to extra endogenous N losses (Figure 4). Extra endogenous N losses of 0.74 g with 0.16 g lectins kg^{-1} diet and 1.13 g with 0.96 g lectins kg^{-1} diet were found. A mean extra endogenous N loss of 1.18 g g^{-1} lectin kg^{-1} diet may be assumed if a non linear relation between the extra endogenous ileal N loss and the dietary lectin content exist. This means that a diet containing 1 g lectin kg^{-1} may have an extra endogenous protein loss, equivalent to at least 7.4 g protein kg^{-1} DMI.

The consequences of dietary lectins on apparent and true ileal digestibility of protein are given in Figure 6. A small effect of dietary lectin level was found for true protein digestibility. The lectins caused an extra endogenous protein loss and caused a clear reduction in the apparent ileal protein digestibility in pig.



FIGURES 5 and 6. The effect of dietary soybean lectin level (g lectins kg^{-1}) on extra endogenous ileal protein loss (g kg^{-1} dry matter intake) and on apparent and true ileal protein digestibility (%) in pigs, respectively.

The impact of the endogenous losses on maintenance requirements can be demonstrated on the basis of a theoretical example. Feeding a 15 kg liveweight pig 600 g/day a diet with 16% crude protein and a dry matter content of 88%, the daily protein and dry matter intake would be 96 g and 528 g, respectively. With an apparent ileal digestibility of 80% 76.8 g crude protein would be available daily for the animal. It should be assumed that the administered diet further contains 100 g NDF kg^{-1} DM, 0.5 g trypsin inhibitor activity kg^{-1} diet and 0.3 g lectins kg^{-1} diet. Further, it is to be assumed that the extra endogenous ileal N is equivalent to the N required for maintenance. According to Fuller et al. (1989) the N requirement for maintenance appears to be 268 mg N kg^{-1} liveweight$^{0.75}$ in pigs. The consequences of including various dietary factors on absolute and relative extra endogenous N losses compared to the N requirement for maintenance are given in Table 3.

TABLE 3. The effects of dietary factors on extra nitrogen requirements for maintenance.

	Nitrogen requirement for			
	Maintenance	Dietary NDF[1]	Dietary TIA[2]	Dietary Lectins[3]
Gram day^{-1}	2.04	0.58	0.40	0.35
Relative to maintenance (%)	100	28.4	19.6	17.2

[1] Extra endogenous ileal N (g kg^{-1} DMI) = -0.120 + 0.007 x g NDF kg^{-1} diet.
[2] Extra endogenous ileal N (g kg^{-1} DMI) = -0.017 + 0.841 x g TIA kg^{-1} diet.
[3] Extra endogenous ileal N (g kg^{-1} DMI) = 1.18 x g Lectins kg^{-1} diet.

The protein requirement for maintenance amounts to about 17% of the daily available protein under these conditions. Assuming that there is an additivity of these tested dietary factors, the inclusion of all of these aforementioned dietary factors will increase the relative amount necessary for maintenance to about 27% of the daily available protein. Per g dietary factor increase trypsin inhibitors and lectins mainly the protein (N x 6.25) costs for extra maintenance requirement. This means that there is less dietary protein available for growth, and secondly that the wastage in faecal and urinary N will also be increased.

Possibilities to reduce endogenous ileal protein losses

As shown in the present study there are a number of dietary factors which may increase endogenous protein (N x 6.25) losses. This may lead to increased protein requirements for maintenance and reduced efficiency of protein gain. As a result, the feed efficiency will be negatively influenced, the production costs will be increased and urinary and faecal N losses will be increased. Therefore, it is very important to find ways to reduce those dietary factors which increase the endogenous ileal protein loss in pigs. Recently Gatel (1993), indicated that this can take place at different levels; plant breeding, preservation of feedstuffs, feedstuffs selection and feed processing. Possible approaches include the modification of the digestive environment to enhance digestive functions of the animal and enzymatic treatment of feedstuffs. Successful application of these treatments to reduce endogenous ileal protein losses, depends on the dietary factor to be influenced.

Implications

The apparent ileal protein (N x 6.25) digestibility, is mainly affected by the excretion of endogenous protein. Animal and dietary factors are responsible for variation in endogenous protein losses. As demonstrated in this study, various dietary factors effect the apparent ileal protein digestibility negatively, and depending on the level of their dietary inclusion they can also reduce the true digestibility. Continuous high losses of endogenous protein of a specific source can cause an imbalance of available amino acids. In the first instance, the animal will try to compensate for these losses by reducing the protein gain. When the endogenous protein losses are extremely high, i.e. with high dietary trypsin inhibitor content, the digestive process may be disturbed. For example, with a high trypsin inhibitor content in the diet, the pig cannot produce enough pancreatic enzymes to compensate for the inhibition of these enzymes (Le Guen, 1993). Due to this effect the enzymatic hydrolysation of dietary protein is effected negatively which results in a lower true protein digestibility. The results obtained are important to upgrading the nutritional quality of raw feedstuffs. They demonstrate the importance of reducing or inactivating those dietary factors that cause an increased endogenous loss. For example, in most raw feedstuffs the true protein digestibility is already high (Huisman et al., 1993). In many cases, the apparent protein digestibility is low due to increased losses of endogenous protein.

The ileal digestibility of different batches of one feedstuff has often been found to be different. As shown in this study, knowledge about the dietary contents of NDF, trypsin inhibitors and lectins enables a better evaluation of the feedstuffs. Even small variation may cause serious effects. Therefore it is important to know more about the effects of dietary factors on physiological responses in the animal. This information is important for the attempts to improve the nutritional quality of raw materials by reduction of these factors hampering an optimal digestibility and availability of nutrients.

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SUMMARY

ZUSAMMENFASSUNG

SAMENVATTING

SUMMARY

The determination of endogenous protein (N x 6.25) and amino acid losses from the gastro-intestinal tract of the pig, is of fundamental importance in nutritional science. Estimates of endogenous losses are used for the correction of apparent digestibility coefficients to true values, and these estimates form a major part of requirements for maintenance. When non digested protein is corrected for endogenous losses this gives the true digestibility. The calculated true digestibility values are an indicator of the potential value of protein in the feedstuff. The endogenous losses on the other hand, represent the investment of the pig in order to handle this feedstuff. For maintenance of minimal functionality of body processes of the pig, there is always a small basal loss of endogenous protein. Depending on the properties directly related to the pig and to the diet fed, additional endogenous protein may be lost. It is generally known that various dietary factors may cause additional endogenous losses in the pig. In this thesis, endogenous nitrogen (N) losses at the terminal ileum, as affected by some purified dietary factors, were studied in young growing pigs using the ^{15}N-isotope dilution method. The applied levels of purified dietary factors in the experiments presented in this thesis, are representative of the levels of feedstuffs used in practice.

The ^{15}N-isotope dilution method is a valuable method for determining relative endogenous ileal nitrogen losses in the pig. This method can be used for all kinds of dietary protein sources fed. It was found that continuous infusion of daily levels of 3 mg or 30 mg ^{15}N-leucine kg^{-1} liveweight to the pig, did not affect estimation of endogenous ileal N losses. This allowed a comparison of data obtained with different levels of ^{15}N-leucine infusion (Chapter 1). To determine endogenous ileal N losses with the ^{15}N-isotope dilution method as presented in Chapters 2, 4, 5, and 6, daily ^{15}N-leucine infusion rates of 5.04 mg kg^{-1} liveweight were used. Compared to results from the literature, a lower infusion level was used. Reduction of this daily amount of stable isotope infused, considerably reduced costs. Steady state conditions of the ^{15}N enrichment in the precursor N-pool were observed after 5 days of continuous infusion (Chapter 1, 2, 6). Investigation of the ^{15}N- enrichment excess of various body N-pools showed similarity between the pools (Chapter 1). This showed that the TCA-soluble blood plasma N, was a valid pool for the determination of endogenous ileal N using the ^{15}N-isotope dilution method. It could be concluded that variation in the estimated endogenous N loss was mainly caused by diet-dependent ^{15}N-enrichment of the ileal digesta N, and not of the body N-pool (Chapter 1). Similar results were found by determining endogenous ileal N losses using ^{15}N-isotope dilution, and the peptide alimentation ultrafiltration methods (Chapter 2). This was a further indication of the validity of the ^{15}N-isotope dilution method.

In the present study, neutral detergent fibre (NDF) isolated from wheat bran, isolated trypsin inhibitors (TI) and lectins (LE) from soya, each at different levels, were used to investigate the effects on endogenous ileal nitrogen losses in pigs using the ^{15}N-isotope dilution method.

Different levels of NDF inclusion, as well as different NDF source were used. The inclusion of water-insoluble fibre (NDF) in the pigs' diet, led to an increase in the endogenous ileal N loss (Chapter 3 and 4). With an increasing content of dietary NDF, additional endogenous N passed the terminal ileum of the pig. The increase in additionally excreted endogenous ileal protein was found to be linear with increasing amounts of purified NDF from wheat bran (Chapter 3). It was also found that dietary NDF increased the ileal losses of undigested dietary protein (Chapter 3). This increase was explained by the increase of undigested dietary NDF bound N in ileal digesta.

To investigate the relationship between the source of dietary NDF and endogenous ileal N excretion, various fibre sources were included in a diet at similar dietary NDF level (Chapter 4). It was found that purified NDF from wheat bran and sun flower hulls with the same amount of NDF, induced similar endogenous ileal N losses. The dietary inclusion of whole wheat bran showed that apart from the NDF, there are some other factors, possibly water soluble carbohydrates, which induced an extra endogenous N loss (Chapter 4).

In peas, common beans and soybeans, both trypsin inhibitors and lectins are present at such levels that they may be considered important. Therefore, the studies presented in Chapter 5 and 6 of this thesis focused on the effect of trypsin inhibitors and lectins on endogenous protein passing the terminal ileum in pigs. Trypsin inhibitors and lectins isolated from soybean were used, because of the importance of soybeans in animal nutrition.

It was found that trypsin inhibitors in the diet led to an increase in the endogenous ileal N loss. Moreover, the amount of endogenous ileal N loss was positively related to the dietary trypsin inhibitor level. The dietary trypsin inhibitor content caused an increase in the amount of dietary protein in the terminal ileum (Chapter 5).

Extra endogenous ileal protein losses were found after feeding isolated soybean lectins to pigs. Even a very small amount of ingested lectins, similar to amounts found in well toasted soybeans, caused an increase in endogenous ileal N loss. A further increase of the dietary lectin level led to extra endogenous N losses (Chapter 6).

As shown in this study, knowledge of the dietary contents of NDF, trypsin inhibitors and lectins and their effects on the pig, enables a better evaluation of the protein value of the feedstuffs. The dose response relation was found of ileal N with those ANF's. Knowledge of this kind of dose response is important to adjust livestock production efficiency. Moreover, environmental pollution can be reduced by reducing urinary and faecal N losses.

ZUSAMMENFASSUNG

Die Bestimmung endogener Eiweiß- und Aminosäurenverluste des Magen-Darm-Traktes im Schwein ist von grundlegender Bedeutung für die Ernährungswissenschaft. Endogene Eiweißsekrete bilden einen wichtigen Bestandteil des Erhaltungsbedarfes beim Schwein. Die quantitative Erfassung endogener Eiweißverluste ist eine Voraussetzung, diesen Anteil genauer zu beschreiben. Darüber hinaus erfordert die Berechnung wahrer Verdaulichkeitskoeffizienten des Futtereiweiß die mengenmäßige Bestimmung endogener Eiweißverluste. Diese berechneten Werte der wahren Verdaulichkeit sind ein Indikator des potentiellen Wertes eines Futtermittels. Andererseits repräsentieren endogene Verluste die Aufwendungen des Schweines, mit einem Futtermittel umzugehen.

Verbunden mit der Erhaltung einer minimalen Funktionalität der im Körper des Schweins ablaufenden Prozesse, wird immer ein basaler endogener Eiweißverlust auftreten. Zusätzliche Verluste an endogenem Eiweiß können auftreten in Abhängigkeit von Veränderungen des Schweins sowie des verabreichten Futters. Allgemein bekannt ist, daß verschiedene Futterfaktoren zusätzliche endogene Eiweißverluste beim Schwein verursachen können.

Das Ziel dieser Arbeit ist es, die Einflüsse verschiedener isolierter Faktoren des Futters auf die endogenen Stickstoffverluste (N) am Ende des Dünndarm bei jungen wachsenden Schweinen mittels der ^{15}N-Isotopen-Verdünnungsmethode zu untersuchen. Die in den dazu durchgeführten Versuchen verwendeten Gehalte an isolierten Futterfaktoren sind vergleichbar mit in der praktischen Fütterung genutzten Mischungen.

Die ^{15}N-Isotopen-Verdünnungsmethode ist eine wertvolle Methode zur Bestimmung relativer endogener Eiweißverluste beim Schwein. Diese Methode ist unabhängig vom gefütterten Eiweiß. Es wurde gefunden, daß eine kontinuierliche Infusion täglicher Mengen von 3 mg bzw. 30 mg ^{15}N-Leucin per kg Körpergewicht die endogenen ilealen N-Verluste nicht beeinflußt. Dadurch sind Vergleiche von Daten, gewonnen mit unterschiedlichen ^{15}N-Leucin Infusionsgehalten, möglich (Kapitel 1). Zur Bestimmung endogener ilealer N-Verluste, mittels der ^{15}N-Isotopen-Verdünnungsmethode, enthalten in den Kapiteln 2, 4, 5 und 6, wurden tägliche ^{15}N-Leucin Infusionsmengen von 5.04 mg per kg Körpergewicht verwendet. Verglichen mit Literaturdaten sind diese Infusionsraten niedriger, was eine beachtliche Verminderung der Kosten mit sich bringt. Bereits nach 5 Tagen kontinuierlicher Infusion wurde die Einstellung einer konstanten ^{15}N-Anreicherung im "Precursor"-Pool beobachtet (Kapitel 1, 2 und 6). Untersuchungen der ^{15}N-Anreicherungen verschiedener Körper-N-Pools zeigten eine Übereinstimmung zwischen den Pools (Kapitel 1). Dies unterstützt die Prämisse, daß TCA-lösliches Blutplasma-N ein berechtigter "Precursor"-Pool zur Bestimmung des

endogenen ilealen N mittels der ^{15}N-Isotopen-Verdünnungsmethode ist. Es wurde des weiteren gefunden, daß Veränderungen von endogenen N-Verlusten hauptsächlich durch nahrungsabhängige ^{15}N-Anreicherungen des ilealen Digesta-N, und nicht des Körper-N-Pools, verursacht wurden (Kapitel 1).
Vergleichbare endogene ileale N-Verluste wurden unter Verwendung der ^{15}N-Isotopen Verdünnungs- und der "Peptide Alimentation Ultrafiltration"-Methode gefunden (Kapitel 2). Dies kann als ein weiterer Beweis der berechtigten Anwendung der ^{15}N-Isotopen-Verdünnungsmethode zur Bestimmung des Anteils endogenen N am Ende des Dünndarmes beim Schwein interpretiert werden.

Mit Hilfe der ^{15}N-Isotopen-Verdünnungsmethode wurden in der vorliegenden Arbeit die Einflüsse verschiedener Gehalte an NDF, isoliert aus Weizenkleie, Trypsininhibitoren (TI) und Lectinen (LE), isoliert aus Soja, auf die endogenen ilealen N-Verluste beim Schwein untersucht.

Der Einfluß verschiedener NDF-Konzentrationen und -Quellen wurde untersucht. Die Aufnahme wasserunlöslicher Fasern (NDF) durch das Schwein über die Nahrung, führt zu einer Erhöhung der endogenen ilealen N-Verluste (Kapitel 3 und 4). Ein steigender Anteil von NDF im Futter verursachte einen zusätzlichen endogenen N-Strom an Ende des Dünndarms beim Schwein. Wie die Untersuchungen zeigten, besteht ein linearer Zusammenhang zwischen der zusätzlichen endogenen ilealen Eiweißausscheidung und des Gehaltes an Futter-NDF, isoliert aus Weizenkleie (Kapitel 3). Darüber hinaus wurde gefunden, daß die Erhöhung des Gehaltes an Futter-NDF ebenfalls eine Erhöhung des nicht verdauten Futtereiweißes am Ende des Dünndarmes verursachte (Kapitel 3). Diese Zunahme wurde mit einer Erhöhung des unverdauten Nahrungseiweißes, welches an NDF gebunden war, erklärt. Zur Untersuchung des Einflusses verschiedener Quellen von Futter-NDF auf endogene ileale N-Ausscheidungen wurden verschiedene Faserquellen so dem Futter zugefügt, daß die verwendeten Mischungen einen einheitlichen NDF-Gehalt aufwiesen (Kapitel 4). Es wurde gefunden, daß aus Weizenkleie isoliertes NDF und die Schalen von Sonnenblumenkernen, mit ähnlichen NDF-Gehalten, vergleichbare endogene ileale N-Verluste verursachten. Darüber hinaus zeigte die Verwendung von intakter Weizenkleie, daß neben NDF noch andere Faktoren, möglicherweise wasserlösliche Kohlenhydrate, existieren, die zusätzliche endogene N-Verluste verursachen (Kapitel 4).

In Erbsen, Bohnen und Sojabohnen sind Trypsininhibitoren und Lectine in solchen Konzentrationen vorhanden, daß sie als bedeutend einzuschätzen sind. Dies leitete dazu, Untersuchungen zum Effekt von Trypsininhibitoren und Lectinen auf die endogenen Eiweißverluste am Ende des Dünndarms beim Schwein durchzuführen (Kapitel 5 und 6). Auf

Grund der Bedeutung von Sojabohnen in der Tierernährung wurden Trypsininhibitoren und Lectine, isoliert aus Sojabohnen, verwendet.
Die Untersuchungsergebnisse zeigten, daß Trypsininhibitoren, verabreicht über das Futter, zu einer Zunahme der endogenen ilealen N-Verluste führten. Darüber hinaus wurde festgestellt, daß der endogene ileale N-Verlust positiv mit dem Gehalt von Trypsininhibitoren im Futter korreliert. Außerdem verursachten die im Futter enthaltenen Trypsininhibitoren eine Zunahme ilealer Ausscheidungen unverdauten Nahrungseiweißes (Kapitel 5).
Zusätzliche endogene ileale Eiweißverluste beim Schwein wurden ebenfalls nach der Fütterung isolierter Soja-Lectine gefunden. Selbst sehr geringe, mit dem Futter zugefügte Lectine-Mengen, vergleichbar mit Mengen die in gut getoasteten Sojabohnen enthalten sind, verursachten eine Zunahme der endogenen ilealen N-Verluste (Kapitel 6).

Wie an Hand dieser Untersuchungen deutlich aufgezeigt wird, ermöglicht die Kenntniss der Gehalte an NDF, Trypsininhibitoren und Lectinen im Futter, sowie deren Einflüsse auf Verdauungsprozesse des Schweines, eine bessere Beurteilung des Eiweißwertes der Futtermittel. Eine gehaltsabhängige Beziehung zwischen ilealen N und den untersuchten ANF's wurde gefunden. Das Wissen dieser Zusammenhänge ist eine wichtige Voraussetzung für die Gestaltung einer effizienten Tierproduktion. Darüber hinaus ist mittels einer Verminderung der Stickstoffausscheidung der Tiere über Urin und Kot eine Verminderung der Umweltbelastung möglich.

SAMENVATTING

Voor de ontwikkelingen in de diervoeding is het van groot belang de endogene verliezen aan stikstof en aminozuren in het maagdarmkanaal te kennen.
In de evaluatie van de eiwitwaardering gaat men steeds meer over van de klassieke bepaling van schijnbare verteerbaarheid naar de bepaling van de ware verteerbaarheid met name van stikstof. Ware verteerbaarheid vereist kennis van de endogene verliezen. Endogene verliezen aan stikstof kunnen gezien worden als deel van de onderhoudsbehoefte. Beide aspekten a) onderhoudsbehoefte van het dier aan eiwit en b) ware verteerbaarheid van voedseleiwit vereisen de bepaling van endogene eiwit op het einde van de dunne darm. In dit proefschrift is het onderzoek naar de invloed van enkele componenten op de hoeveelheid endogene eiwit beschreven zoals dat verricht is met jonge varkens. De endogene verliezen aan stikstof tijdens het verteringsproces in maag en dunne darm, met name die nog aanwezig zijn op het eind van de dunne darm, zijn gemeten. Als voerfaktoren zijn Neutral Detergent Fibre (NDF), Trypsine Inhibitor (TI) en Lectine (Le) gebruikt. Rantsoenen met verschillende hoeveelheden van deze componenten zijn aan jonge varkens gevoerd en er is gemeten hoe deze componenten de endogene verliezen en de ware verteerbaarheid beïnvloeden. Ware verteerbaarheid kan dan gezien worden, als max vertering van N in dat rantsoen, door het dier. Dit is min of meer een waarde voor de potentiële max verteerbaarheid. Endogene verliezen kunnen gezien worden als verliezen van lichaamseiwit tijdens het verteringsproces en die niet opnieuw geabsorbeerd worden.
In dit proefschrift is onderzoek verricht met gezuiverde NDF, TI en Le. Deze zijn in verschillende hoeveelheden aan een standaardrantsoen toegevoegd. Middels de ^{15}N isotopen techniek is gemeten hoeveel endogene N verliezen door deze componenten werden geïnduceerd. De hoogte van de componenten is representatief voor gehalten zoals die in normale rantsoenen voorkomen.

In de eerste serie proeven is de methodiek van meten nader gevalueerd. Ofschoon de ^{15}N techniek reeds in veel onderzoeken is gebruikt om endogene verliezen in maagdarmkanaal te meten is deze nog steeds niet erg goed geevalueerd.
Als eerste aspekt van de methodiek is de dosis tijdens infusie getest. Bij deze techniek wordt gedurende enkele dagen continue ^{15}N geïnfuseerd in een dier en na enkele dagen wordt aangenomen dat het ^{15}N gehalte in de TCA oplosbare N fractie in bloedplasma gelijk is aan dat van de precursor waaruit endogene eiwit wordt gemaakt. Tot nu toe werd veelal 40 mg ^{15}N per dag per kilogram lichaamsgewicht geïnfuseerd. Er werd getest of er een verschil is in de schatting van endogene eiwit bij 3 en 30 mg. Beide hoeveelheden gaven geen verschil in

schatting (Hoofdstuk 1). Daarom is in vervolgonderzoek met een dosis van 5 mg ^{15}N gewerkt (Hoofdstuk 2,4,5,6). Bovendien bleek dat de ^{15}N verrijking van het lichaam (gemeten in de TCA opl. bloedplasma) na ongeveer 5 dagen een steady state status heeft bereikt (Hoofdstuk 1, 2 en 6).

Als 2e methodisch aspekt is nagegaan of in organen die de endogene eiwitten produceren, pancreas en darmwand, eenzelfde verrijking van ^{15}N optreedt. Het bleek dat zowel bloed, darm als pancreas practisch dezelfde labelling hadden en dat de rantsoensamenstelling deze labelling niet erg beïnvloede (Hoofdstuk 2).

Als 3e controle op de methode werd een vergelijking gemaakt tussen de endogene verliezen gemeten met ^{15}N en die bepaald met de peptide alimentatie ultrafiltratie methode. Bij deze laatste wordt alleen voereiwit bestaand uit di- of tripeptide (EHC, enzymatisch gehydroliseerde caseïne) gebruikt. Het eiwit aanwezig op het eind van de dunne darm en grooter dan 10 kDa is dan van het dier afkomstig. Beide methoden, ^{15}N en EHC, gaven gelijke hoeveelheden endogene verliezen (Hoofdstuk 2). Er werd daarom geconcludeerd dat de ^{15}N methode een valide methode is om endogene verliezen te meten.

In dit onderzoek werden 3 voedingscomponenten bestudeerd die volgens de literatuur invloed kunnen hebben op de schijnbare vertering van eiwit. Er werd NDF geïsoleerd uit tarwezemelen, de andere componenten waren geïsoleerde Trypsine Inhibitor uit soja (TI) en Lectine (Le) geïsoleerd uit soja. Elk van deze 3 componenten werd in verschillende hoeveelheden gebruikt en de endogene N verliezen in de dunne darm werden gemeten.

De opname van geïsoleerde NDF in het rantsoen leidde zowel tot extra onverteerd voedsel eiwit als tot extra endogeen eiwit (Hoofdstuk 3 en 4). Met toename aan NDF nam de endogene ileale N hoeveelheid lineair toe (Hoofdstuk 3). Als verklaring voor de toename aan onverteerd voedsel kan mogelijk de binding van voedseleiwit aan NDF als verklaring dienen. Wanneer NDF in tarwezemelen met een gelijke hoeveelheid daaruit gezuiverde NDF werden vergeleken, resulteerde dit in een grotere hoeveelheid endogene verliezen by tarwezemelen. Er zijn dus nog andere faktoren in tarwezemelen die de endogene verliezen verhogen. Daarentegen gaven zonnebloem hullen (met gelijke NDF) en gezuiverde tarwe NDF gelijke waarden (Hoofdstuk 4). NDF bevattende grondstoffen geven dus waarschijnlijk minstens evenveel endogene verliezen als die hier gemeten zijn, of hogere waarden.

Trypsine Inhibitoren (TI) in erwten, Phaseolus bonen en soja zijn in deze producten op een dusdanig niveau aanwezig dat ze belangrijk kunnen zijn als veroorzaker van endogene verliezen. Trypsine Inhibitor (TI) verhoogde de endogene (N) verliezen in de dunne darm van jonge varkens als functie van TI hoeveelheid. Ook werd een toename van onverteerd voedsel eiwit gevonden. Kennelijk is de compensatie door extra secretie van verteringsenzymen bij

aanwezigheid van TI niet volledig (Hoofdstuk 5).
Als 3e component is lectine uit soja onderzocht. Zelfs een kleine hoeveelheid lectine (gelijk aan die in getoaste soja bonen) gaf al extra endogene eiwitverliezen. Toename van dit niveau gaf ook meer endogene N (Hoofdstuk 6). Uit deze serie proeven kan worden geconcludeerd dat endogene eiwitten een zeer groot deel van de schijnbare onverteerde N op eind van ileum uitmaakt (minimaal 50%). Alle onderzochte componenten NDF, TI en Le verhoogden de endogene secretie. Bovendien leidde NDF en TI ook tot een afname van de ware verteerbaarheid van N. Produkten met niet gezuiverde NDF kunnen een hoger endogeen verlies geven dan de zuivere NDF. Het blijkt dat alle drie de onderzochte componenten (ANF's) een dose response relatie hebben met endogeen N verlies. Deze relatie is essentieel om te bepalen hoeveel verliezen deze ANF's opleveren.

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