NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

THE UNIVERSITY OF ALBERTA

ENDOGENOUS PROTEIN IN DIGESTIBILITY STUDIES IN PIGS

by

CORNELIS FRANCISCUS MARIA DE LANGE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1989

ISBN 0-315-52924-5

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: CORNELIS FRANCISCUS MARIA DE LANGE

TITLE OF THESIS: ENDOGENOUS PROTEIN IN DIGESTIBILITY STUDIES IN PIGS

DEGREE: DOCTOR OF PHILOSOPHY

YEAR THIS DEGREE GRANTED: SPRING 1989

(SIGNED) .....

PERMANENT ADDRESS:

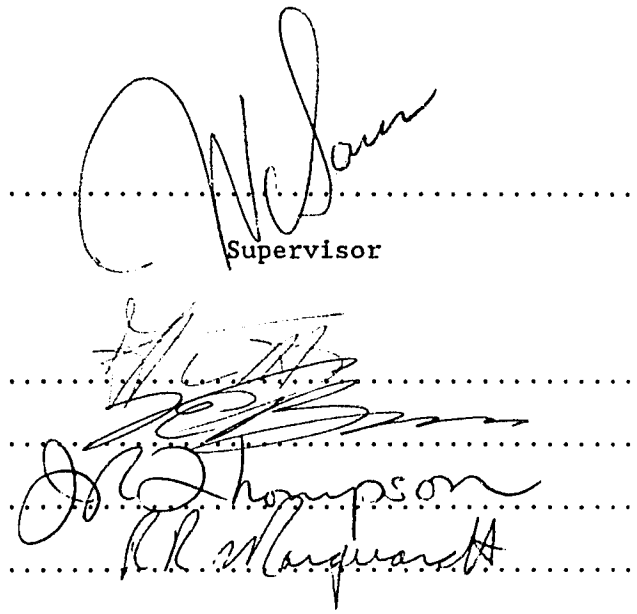
...Driesledenweg..11.....
...1662...BE...De...Weere....
...The....Netherlands.....

DATED 21...Dec. 1988

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ENDOGENOUS PROTEIN IN DIGESTIBILITY STUDIES IN PIGS submitted by CORNELIS FRANCISCUS MARIA DE LANGE in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Animal Nutrition.



Supervisor

Date 21 Dec 1988

DEDICATION

To my wife,

Deirdre

ABSTRACT

Three experiments were conducted with growing pigs, fitted with simple T-cannulas at the distal ileum, to study factors that may affect the recovery of endogenous protein in digesta collected from the distal ileum. In the first experiment, barrows were fed four different protein-free diets. Diet I represented a standard, cornstarch-based, protein-free diet. In diets II, III and IV, four percent pectin, seven percent additional Alphafloc (a source of cellulose) and ten percent additional canola oil were included, respectively. The recovery of endogenous protein at the distal ileum was affected by the composition of the protein-free diet ($P<.05$): 19.8, 24.0, 22.5, and 20.0 g per kg dry matter intake for diets I, II, III and IV, respectively. Of the endogenous amino acids, only the recoveries of arginine, glycine and proline were influenced by the diets ($P<.05$).

In the second experiment, barrows were fed a standard protein-free diet and simultaneously administered a saline solution or a well-balanced mixture of amino acids parenterally. The intravenous administration of amino acids reduced ($P<.05$) the recovery of endogenous protein in digesta collected from the distal ileum: 12.7 versus 18.5 g per kg dry matter intake. Of the endogenous amino acids, only the recovery of proline was affected ($P<.05$).

In the third experiment, the ^{15}N-isotope dilution technique was used to determine the recovery of endogenous protein in digesta collected from the distal ileum in gilts fed protein-containing diets. It was concluded that the results obtained with the ^{15}N-isotope

dilution technique were probably more reliable than those obtained with the alternative ^{15}N-leucine and ^{15}N-isoleucine isotope dilution techniques. The amounts of endogenous protein recovered at the distal ileum, as determined with the ^{15}N-isotope dilution technique, were 25.5, 30.5, 27.4 and 27.7 g per kg dry matter intake in diets in which soybean meal, canola meal, wheat and barley were included as the sole protein source, respectively. The directly determined real ileal protein digestibilities were 97.5, 84.1, 99.0 and 94.2% for the soybean meal, canola meal, wheat and barley diets, respectively. In addition, real ileal amino acid digestibilities were determined. The real ileal protein and amino acid digestibilities were higher than the indirectly calculated true ileal protein and amino acid digestibilities, based on feeding protein-free diets to determine the endogenous protein and amino acid recoveries at the distal ileum.

ACKNOWLEDGEMENTS

I would like to thank Dr. M. A. Price and Dr. R. T. Hardin, Chairman and former Chairman of the Department of Animal Science, for placing the facilities of the department at my disposal.

I wish to thank Dr. W. C. Sauer, Associate Professor of Swine Nutrition, for his advice and support throughout my Ph. D. program.

I am indepted to Dr. W. B. Souffrant from the Oskar Kellner Institute in Rostock, G. D. R., for his assistance and suggestions during the last experiment.

I would like to thank the academic and support staff of the department for their guidance throughout my studies at the University of Alberta, especially Dr. V. E. Baracos and Dr. J. R. Thompson for their contributions during the initial stages of the studies.

I wish to acknowledge many people for their assistance during surgery, the intensive parts of the studies and in chemical analyses, especially, Dr. R. Mosenthin, Brenda Kozak, Charlane Shellian, Mike Dugan, Irma Schutte, Renee Polziehn and Frank Maenhout. The work of Kelvin Lien in taking care of the GCMS analyses was greatly appreciated.

Many thanks are expressed to my mother, father and family for their continuous support and advice troughout my years of education.

Lastly, I wish to thank Deirdre, my wife, for her patience, encouragement and understanding throughout my studies.

Financial support for these studies was provided by the Agricultural Research Council of Alberta and the Natural Sciences and

Engineering Research Council. The ^{15}N-isotopes for the studies were provided by the Oskar Kellner Institute. These are gratefully acknowledged.

Table of Contents

List of Tables

List of figures

I. INTRODUCTION

An important determinant in the accurate formulation of diets for pigs is an accurate knowledge of the availability of the amino acids in the individual feedstuffs: the proportion of dietary amino acids that are absorbed in a form suitable for utilization (Sauer and Ozimek, 1986). Digestibility is related to availability; however, the meanings of these terms are different. Digestibility of dietary amino acids should be defined as the proportion of dietary amino acids that disappear from the gastro-intestinal tract. The fates of the amino acids that disappear from the gastro-intestinal tract are not considered in the estimation of the digestibility of amino acids. Three methods can be used to estimate the digestibility in vivo: the ileal and fecal analysis method (Tanksley and Knabe, 1984; Sauer and Ozimek, 1986) and the quantitative absorption method (Rerat, 1985). Each of these methods will give different estimates of the digestibility of amino acids (Darcy-Vrillon et al., 1985). Digestibilities determined by fecal analysis are confounded by bacterial activity in the large intestine, resulting in amino acid synthesis and disappearance without benefit to the animal. Digestibilities determined by collecting digesta from distal ileum are not influenced by the microbial activity in the large intestine but remain confounded by the residual non-absorbed endogenous protein fraction. The quantitative absorption method takes into account both the recycled endogenous nitrogen and alterations that can occur in intestinal tissue during transport to portal blood. Intestinal tissue is metabolically very active as indicated by the its high rates of

protein turnover and heat production (Koong et al., 1985; Low, 1985).

The ileal analysis method, at present, is preferred for the evaluation of amino acid digestibilities in feedstuffs. The observed values are referred to as apparent ileal digestibilities when the digestibility coefficients are not corrected for the endogenous amino acid recoveries. Apparent protein or amino acid digestibilities are, therefore, affected by both the true protein or amino acid digestibilities and the recoveries of endogenous protein or amino acids, respectively.

Two methods have traditionally been used to determine the endogenous amino acid recoveries in feces or in digesta collected from the distal ileum (Carlson and Bayley, 1970). In the first and most commonly used method ("direct method"), the endogenous recoveries of amino acids are determined by measuring the levels of amino acids in feces or ileal digesta of animals fed a diet devoid of protein. In the second method ("regression method"), the endogenous amino acid recoveries are determined by aid of regression to zero protein intake in which a series of diets that contain graded levels of protein are fed. Apparent amino acid digestibilities, corrected for endogenous amino acid recoveries, determined with the direct or regression method, have traditionally been referred to as true digestibilities. However, the introduction of a new method, the ^{15}N-isotope dilution technique, has shown that the values observed with these traditional methods might be far from "true" (Krawielitzki et al., 1977).

The ^{15}N-isotope dilution technique is based on the assumption that, by labelling the protein in the body with ^{15}N, a differentiation can be made between the non-digested dietary and

endogenous protein (Krawielitzki et al., 1977; Souffrant et al., 1981, 1986). Endogenous rather than dietary protein is labelled in these studies. Labelled amino acids that are administered orally will be absorbed rapidly, incorporated into endogenous protein and secreted into the digestive tract within several hours (Simon et al., 1983), complicating the differentiation between endogenous and dietary protein in the gastro-intestinal tract. The most efficient way of labelling body protein is via continuous intravenous infusion of labelled amino acids, resulting in a constant enrichment excess in the free plasma amino acids (Souffrant et al., 1981). ^{15}N-glycine, the cheapest source of ^{15}N-amino acids, however, can not be used for this purpose. Glycine is selectively secreted with bile acids into the digestive tract (Souffrant et al., 1981). ^{15}N-leucine, therefore, is used as an alternative. Furthermore, because of transamination the ^{15}N infused with leucine will also appear in other amino acids and urea in the blood plasma (Matthews et al., 1979; Harper et al., 1984). Since the amino acids for protein synthesis are derived from the free amino acid pool, the label will also be incorporated into body protein, including endogenous protein which is secreted into the digestive tract. Recent studies with the ^{15}N-isotope dilution technique showed that the enrichment excess in the trichloroacetic acid (TCA)-soluble fraction of blood, which contains the free plasma amino acids, to be similar to the enrichment excess in endogenous protein that was secreted into the digestive tract when ^{15}N-leucine was infused continuously (Souffrant et al., 1986). According to these studies, the TCA-soluble fraction of blood can be considered the precursor pool (the pool from which the amino acids are derived) for synthesis of

endogenous protein that is secreted into the digestive tract when ^{15}N-leucine is used to label endogenous protein.

When the ^{15}N-enrichment excess in endogenous protein has been determined, from the enrichment excess in the precursor pool, and the total amount of label excreted with feces or passing at the distal ileum, the total amount of endogenous protein can be calculated. A clear distinction can then be made between non-digested dietary and endogenous protein.

The observed true protein and amino acid digestibilities, determined with the ^{15}N-isotope dilution technique, were vastly different from the values obtained with the traditional methods that a new term was introduced, namely "real" amino acid digestibility (Krawielitzki et al., 1977; Low, 1982). The difference between real and true protein or amino acid digestibilities was probably largely due to the fact that the endogenous protein excretion was positively related to the protein content of the diet (Krawielitzki et al., 1977). In addition, protein free-diets are usually composed of purified ingredients, which do not contain components like pectins, lignins or tannins. These components are often present in protein-containing feedstuffs and thus present in protein-containing diets. The presence of components, other than protein, in the protein-containing diets may also affect the recovery of endogenous protein and amino acids at the distal ileum in pigs.

The ^{15}N-isotope dilution technique only provides information on the total amount of the endogenous protein recovery, not on the recovery of each of the individual amino acids. The real digestibility of the individual amino acids is very important as under practical

conditions only a few amino acids in the diet are limiting for optimal performance (Sauer and Ozimek, 1986). If assumptions are made concerning the amino acid composition of endogenous protein recovered at the distal ileum or in feces, real amino acid digestibilities can then be calculated from apparent amino acid digestibilities and real protein digestibilities as observed with the ^{15}N-isotope dilution technique. However, there is a scarcity of information on the variation and, in particular, the factors affecting the variation in the amino acid composition of endogenous protein (Wuensche et al., 1987). This variation should be determined prior to the use of the ^{15}N-isotope dilution technique to estimate real amino acid digestibilities.

A major criticism on the ^{15}N-isotope dilution technique is that all the N-containing substances in the TCA-soluble fraction of blood are not uniformally labelled with ^{15}N, when ^{15}N-leucine is infused continuously (Matthews et al., 1979). Systematical errors may be introduced when enrichment excess in total N in the TCA-soluble fraction of blood is used as the indicator for the enrichment excess in endogenous protein. The latter consideration may be important when the relative contribution of different amino acids and other small N-containing molecules, such as urea, to endogenous protein is different from the relative contribution to total N in the TCA-soluble fraction of blood. An alternative method would be to measure the ^{15}N-enrichment excess in leucine in the TCA-soluble fraction of blood and in leucine in digesta or feces using gas chromatography-mass spectrometry (Matthews et al., 1979). If the contribution of endogenous leucine to endogenous protein is assumed constant, then the

recovery of endogenous protein in ileal digesta or feces can be determined using an ^{15}N-leucine isotope dilution technique. In this manner an alternative, newly introduced ^{15}N-leucine isotope dilution technique could be compared to the ^{15}N-isotope dilution technique. Such an isotope dilution technique could be used simultaneously for other amino acids that show incorporation of ^{15}N due to transamination.

The objectives of the present studies were as follows:

(1) to determine some of the factors which may affect the recovery of endogenous protein and amino acids at the distal ileum and feces in pigs fed protein-free diets,

(2) to determine the real ileal protein and amino acid digestibilities in some commonly used feedstuffs for pigs using the ^{15}N-isotope dilution technique, and

(3) to compare the ^{15}N-isotope dilution technique to alternative ^{15}N-leucine and ^{15}N-isoleucine isotope dilution techniques for determining the recovery of endogenous protein in ileal digesta of pigs.

A. BIBLIOGRAPHY

Carlson, K. H. and H. S. Bayley. 1970. Nitrogen and amino acids in the feces of young pigs receiving a protein-free diet and diets containing graded levels of soybean meal or casein. J. Nutr. 100: 1353.

Darcy-Vrillon, B., W. B. Souffrant, J. P. Laplace, A. Rerat, G. Gebhardt, P. Vaugelade and J. Jung. 1985. Digestion of proteins in the pig: ileal and fecal digestibilities and absorption coefficients of amino acids. In: A. Just, H. Jorgensen and J. A. Fernandez (Ed.). Proc. 3rd Int. Seminar on Digestive Physiology in the Pig, Copenhagen: 326.

Harper, A. E., R. H. Miller and K. P. Block. 1984. Branched-chain amino acid metabolism. Ann. Rev. Nutr. 4: 409.

Koong, L. J., C. L. Ferrell and J. A. Nienaber. 1985. Assessment of interrelationships among levels of intake and production, organ size and fasting heat production in growing animals. J. Nutr. 115: 1383.

Krawielitzki, K., T. Volker, S. Smulikowska, H. D. Bock and J. Wuensche. 1977. Weitere Untersuchungen zum Multikompartment-Modell des Proteinstoffwechsels. Arch. Tierernaehr. 27: 609.

Low, A. G. 1982. Endogenous nitrogen evaluation from absorption studies In: Physiology digestive chez le porc, Jouy-en-Josas. Les Colloques de l'INRA. 12: 189.

Low, A. G. 1985. Amino acid use by growing pigs. 1985. In: D. J. A. Cole and W. Haresign (Ed.). Recent developments in pig nutrition. Butterworths, London.

Matthews, D. E., E. B. Ben-Galim and D. M. Bier. 1979. Determination of stable isotope enrichment in individual plasma amino acids by chemical ionization mass spectrometry. Anal. Chem. 51: 80.

Rerat, A., 1985. Intestinal absorption of end products from digestion of carbohydrates and proteins in the pig. Arch. Tierernaehr. 35: 461.

Sauer, W. C. and L. Ozimek. 1986. Digestibility of amino acids in swine: results and their practical application. A review. Livestock Prod. Sci. 15: 367.

Simon, O., T. Zebrowska, H. Bergner and R. Muenchmeyer. 1983. Investigations on the pancreatic and stomach secretion in pigs by means of continuous infusion of ^{14}C amino acids. Arch. Tierernaehr. 33: 9.

Souffrant, W. B., R. Koehler, R. Matkowitz, G. Gebhardt and H. Schmandke. 1981. Ernaehrungsphysiologische Untersuchungen an Schweinen zur Beurteilung von modifizierten Proteinen. Arch. Tierernaehr. 31: 675.

Souffrant, W. B., B. Darcy-Vrillon, T. Corring, J. P. Laplace, R. Koehler, G. Gebhardt and A. Rerat. 1986. Recycling of endogenous nitrogen in the pig. Arch. Tierernaehr. 36: 269.

Tanksley, F. D. and D. A. Knabe. 1984. Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. In: W. Haresign and D.J.A. Cole (Ed.). Recent Advances in Animal Nutrition. Butterworths, London.

Wuensche, J., U. Hermann, M. Meinl, U. Hennig, F. Kreienbring and P. Zwierz, 1987. Einfluss exogener Faktoren auf die praezaekale Naehrstoff und Aminosaeurenresorption ermittelt an Schweinen mit Ileo-Rektal-Anastomosen. Arch. Tierernaehr. 37: 745.

II. THE EFFECT OF FEEDING DIFFERENT PROTEIN-FREE DIETS ON THE RECOVERY AND AMINO ACID COMPOSITION OF ENDOGENOUS PROTEIN COLLECTED FROM THE DISTAL ILEUM AND FECES IN PIGS[1]

A. INTRODUCTION

The ileal analysis method is the preferred method for the determination of amino acid digestibilities in feedstuffs for pigs at present (Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). However, measurements obtained with this method are confounded by non-reabsorbed endogenous amino acids. Using conventional methods, the endogenous amino acids can not be quantified when protein-containing feedstuffs are fed (Carlson and Bayley, 1970).

By aid of the ^{15}N-isotope dilution technique in which endogenous protein, including protein that is secreted into the gastro-intestinal lumen, is labelled, a differentiation can be made between non-digested dietary and endogenous protein in the digestive tract (Souffrant et al., 1986). However, the ^{15}N-isotope dilution technique can only be applied to the determination of the total recovery of endogenous protein, not the recovery of each of the amino acids. On the assumption, however, that the amino acid composition of non-reabsorbed endogenous protein is constant, the true amino acid digestibilities can be derived by calculation from the true protein digestibility.

The objective of the present study was to determine the variation in the recovery and amino acid composition of endogenous protein in

---

[1]A version of this chapter has been accepted for publication. de Lange, C. F. M., W. C. Sauer, R. Mosenthin and W. B. Souffrant. 1988. J. Anim. Sci. (In press).

ileal digesta and feces of pigs fed protein-free diets of differing composition, prior to the use of the ^{15}N-isotope dilution technique in our laboratory.

B. EXPERIMENTAL PROCEDURE

Initially, eight barrows (Lacombe x Yorkshire), with an average body weight of approximately 60 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were slightly modified. The ring at the base of the cannula was replaced by two "wings" with a length of approximately 3 cm. After surgery, the animals were housed individually in stainless steel metabolic crates in a temperature controlled barn (20 to 22°C). Throughout the recovery period, with a duration of at least 10 d, the animals were fed an 18% crude protein starter diet (Sauer et al., 1983). During the first seven d of the recovery period the daily feed allowance was gradually increased until the pigs consumed 1600 g daily.

Following recovery, the animals were fed one of four protein-free diets (Table II.1) according to a Latin square design. They were fed 800 g twice daily, at 0800 and 2000. Water was freely available from a low-pressure drinking nipple.

Each of the diets was formulated to affect the secretion and(or) reabsorption of endogenous protein in a specific manner. Diet I: the control diet, represents a standard-type protein-free diet. Diet II: 4% pectin was included at the expense of cornstarch to stimulate bacterial fermentation, especially in the large intestine (Mosenthin and Henkel, 1983). Diet III: 7% additional (to provide a total of 10%)

Alphafloc (a source of cellulose) was included at the expense of cornstarch. A high level of cellulose has been shown to result in an increase in mucin production (Schneeman et al., 1982), sloughed-off mucosal cells (Bergner et al., 1975) and pancreatic secretion of protein (Zebrowska, 1985). Diet IV: 10% additional canola oil (to provide a total of 13%) was included at the expense of cornstarch. High levels of fat in the diet have been shown to induce an increase in the pancreatic secretion of lipase (Ozimek et al., 1985). Sucrose was added to possibly improve the palatability of the purified diets. Vitamins and minerals were supplemented according to NRC (1979) standards. Chromic oxide (.5%) was included in the diet as the marker for the determination of the recovery of protein and amino acids in ileal digesta and feces.

Each experimental period consisted of 13 d. After a 7-d adaptation period, feces were collected for 3 d at 12-h intervals by aid of anal stimulation. Thereafter, ileal digesta were collected for 2 d continuously with a 24-h interval between the two collection periods according to procedures adapted from Sauer and Thacker (1986). Digesta were collected through soft plastic tubing (4 cm in width), which was attached to the ileal cannula. The lower section of the tubing was kept immersed in a container filled with ice water (4 $^{\circ}$C). Feces and digesta were frozen immediately after collection. Following each experimental period the animals were allowed a recovery period of 8 d during which they were fed an 18% crude protein starter diet (Sauer et al., 1983).

Animals with incomplete feed intake (less than 90% of the daily allowance) or that leaked severely around the cannula were discarded

and replaced by animals of similar background and bodyweight. Three animals were replaced in total.

The animals were sacrificed at the conclusion of the experiment and dissected to observe whether cannulation had caused adhesions or other intestinal abnormalities.

Analytical and Statistical Procedures. After the conclusion of the experiment, feces were pooled per animal and per experimental period. Ileal digesta were pooled per animal for each 24-h collection period. The pooled samples were freeze-dried, ground in a Wiley mill through a 1-mm mesh screen, and thoroughly mixed before samples were taken for further analyses. Analyses for nitrogen and dry matter were carried out according to AOAC (1980). The nitrogen present in the protein-free diets (0.05 to 0.07%) was ignored in the interpretation of the results. Chromic oxide levels in feed, digesta and feces were determined according to Fenton and Fenton (1979). Amino acid analyses were performed following acid hydrolysis in 6N HCl for 24 h using a Beckman 121MB amino acid analyzer[2] (Blackburn, 1968). All analyses were performed in duplicate.

The results were subjected to least square analyses of variance for unequal numbers (Harvey, 1960). Only the main effects (diet, animal, period and collection day) were included in the statistical model. The standard errors of means were calculated using the average number of observations per mean (n=6.5). Least square means for significant treatment differences were compared using the Student Newman-Keuls multiple range test (Steel and Torrie, 1980).

---

[2]Beckman Instruments Inc., Palo Alto, California 94304, USA.

C. RESULTS

Cannulation did not result in intestinal abnormalities in the nine barrows from which valid observations were obtained.

The fecal dry matter digestibilities were higher than the ileal dry matter digestibilities (Tables II.2 and II.3). The difference between ileal and fecal dry matter digestibilities was largest for the pectin containing diet (8.9 percentage units). In addition, less protein was recovered in feces than in ileal digesta.

The addition of fat, pectin, and especially cellulose, depressed ($P<.05$) the digestibility of dry matter in ileal digesta (Table II.2). Only the addition of cellulose reduced ($P<.01$) the dry matter digestibility measured in feces (Table II.3).

The inclusion of pectin increased ($P<.05$) the quantity of endogenous protein recovered in ileal digesta (Table II.2). This increase resulted largely from an increased recovery of proline ($P<.01$) and glycine ($P<.05$), the predominant amino acids in endogenous protein. The levels of most of the other amino acids were also increased, although not significantly ($P>.05$). The addition of cellulose also tended to increase the recovery of endogenous protein and most of the amino acids in ileal digesta; only the recoveries of proline and glycine increased significantly ($P<.05$). The net disappearance of protein in the large intestine, calculated as recovery in ileal digesta minus recovery in feces, was most prominent for the pectin-containing diet (15.0 g per kg dry matter intake), (Tables II.2 and II.3). The disappearance was smallest for the control diet (11.4 g per kg dry matter intake). Of the dispensible amino acids, aspartic acid, glutamic acid, serine, and especially glycine and proline

disappeared to a large extent in the large intestine. Of the indispensible amino acids, the disappearance was most marked for phenylalanine and threonine. A net synthesis was observed for methionine. The differences between ileal and fecal amino acid recoveries were consistent for all four diets (Tables II.2 and II.3).

In feces, only the inclusion of additional cellulose in the diet increased ($P<.05$) the endogenous protein excretion (Table II.3). This increase was associated with an increased ($P<.05$) recovery of all amino acids with the exception of leucine, methionine, phenylalanine, valine, proline, and tyrosine.

The addition of fat did not affect ($P<.05$) the levels of endogenous protein or amino acids, in both ileal digesta and feces (Tables II.2 and II.3).

D. Discussion

The amounts of endogenous amino acids recovered at the distal ileum or excreted in feces were measured in the present experiment. Since a portion of endogenous protein is digested and reabsorbed, the observed values refer to the balance of secretion and reabsorption of endogenous protein (Low, 1982). Protein that disappears in the large intestine is not considered beneficial to the animal (Zebrowska, 1973). Therefore, endogenous amino acids passing at the distal ileum can be considered as losses to the animal. The observed differences in the recovery of amino acids between ileal digesta and feces as well as in ileal and fecal dry matter digestibilities (Tables II.2 and II.3) are largely due to microbial fermentation in the large intestine (Kidder and Manners, 1978; Mosenthin and Henkel, 1983). The net

disappearance of most amino acids and the net synthesis of methionine has also been reported in previous studies (Holmes et al., 1974; Sauer et al., 1977; Wuensche et al., 1979; Taverner et al., 1981) and introduce errors when amino acid digestibilities are determined with the fecal analysis method.

Sauer et al. (1977) discussed the effect of dry matter intake and digestibility on the recovery of endogenous protein in ileal digesta. Because of its mechanical effect, intake of more non-digested materials, primarily derived from fiber, may result in increased endogenous protein losses. The results of the present studies tend to confirm this relationship (Tables II.2 and II.3). Pectin apparently has an additional effect as it results in digesta of a more viscous nature which might induce an increase in the secretion and(or) decrease in the reabsorption of endogenous amino acids in the ileum (Table II.2).

The increased recovery of endogenous protein in ileal digesta was largely due to an increase ($P<.05$) in the recoveries of glycine and proline (Table II.2), which in turn, probably resulted from a relatively large contribution of saliva and(or) bile to endogenous protein. According to Horowitz (1967) and Low (1982), glycoproteins excreted with bile and saliva contain relatively large amounts of proline and glycine. The major function of saliva is to facilitate movement of digesta to the stomach and to initiate digestion of glucose polymers containing α, 1-6 bonds (Kidder and Manners, 1978). Bile plays an important role in the digestion and absorption of dietary fat (Kidder and Manners, 1978). The lower ($P<.05$) glycine recovery in ileal digesta, when the high-fat compared to the pectin diet was fed

(Table II.2), might be attributed to an increase in the reabsorption of bile acids as more dietary fat is absorbed.

The recovery of proline and glycine in ileal digesta was also increased ($P<.05$) when additional cellulose was included in the diet (Table II.2). The effect of cellulose may be mediated through its mechanical effect as well. Fiber has been shown to result in an increase in mucus production (Schneeman et al., 1982) and sloughed-off mucosal cells (Bergner et al., 1975). Fiber is also capable of adsorbing amino acids and peptides, withholding these from re-absorption (Bergner, 1982).

When the recoveries of endogenous amino acids in ileal digesta were expressed as a percentage of total endogenous protein (Nx6.25), small but significant differences ($P<.05$) appeared for all amino acids (Table II.4). The relatively high abundance of proline and glycine in endogenous protein, when the pectin and cellulose-containing diets were fed, depressed the concentration of other amino acids.

The increased ($P<.05$) amount of endogenous protein excreted in feces, when additional cellulose was included in the diet (Table II.3), may be attributed to its mechanical effect as well. However, non-digestible but fermentable carbohydrates will be used as substrates for microbial fermentation, resulting in an increased amount of bacterial protein excreted in feces. Endogenous protein can be used for bacterial protein synthesis. It has been estimated that bacterial protein makes up more than 50 percent of the total protein excreted in feces (Gargallo and Zimmerman, 1981; Meinl and Kreienbring, 1985; Mosenthin, 1987). The increment in the recovery of endogenous protein was related to increments ($P<.05$) in the recoveries of most of the

amino acids (Table II.3). The ratio of concentrations between amino acids was relatively constant; most likely the result of a large contribution of bacterial protein to protein in feces. With the exception of phenylalanine and tyrosine, there were no differences ($P>.05$) in the amino acid composition of endogenous protein (N x 6.25) in feces between treatments (Table II.5).

In other studies with surgically modified pigs, the amount of endogenous protein recovered at the distal ileum varied from 10.0 to 17.5 g protein per kg dry matter intake (Holmes et al., 1974; Sauer et al., 1977; Wuensche et al., 1979; Taverner et al., 1981). Values observed in the present experiment were somewhat higher (Table II.2). The large animal effects ($P<.01$) observed in the present study may have contributed to these differences. Pectin was not included in the diets in the aforementioned studies. The largest amount of endogenous protein was recovered in the ileal digesta of pigs fed the pectin containing diet. There was a trend towards a larger recovery of endogenous protein in ileal digesta when additional cellulose was included in the diet which is in agreement with other studies (Sauer et al., 1977; Taverner et al., 1981).

The amount of endogenous protein recovered in feces and the amino acid composition of endogenous protein recovered in ileal digesta or excreted in feces (Tables II.3 and II.4) falls within the range of values reported in other studies (Holmes et al., 1974; Sauer et al., 1977; Wuensche et al., 1979; Taverner et al., 1981). The content of proline, in particular, and glycine in endogenous protein recovered in ileal digesta varied widely between the aforementioned studies, from 11.9 to 37.0% and from 4.7 to 13.3%, respectively. These differences

explain a major part of the differences in the recovery of endogenous protein in ileal digesta that have been previously reported.

The determination of the true protein and amino acid digestibilities requires information on the quantity of endogenous protein and amino acids. Differences in these quantities are usually not considered in these calculations, although differences between results obtained from different studies exist (Holmes et al., 1974; Wuensche et al., 1979; Sauer et al., 1977; Taverner et al., 1981). These differences can either be of physiological nature or result from differences in experimental design. The effect of diet composition (additional cellulose) on the endogenous amino acid recoveries were studied in only two experiments. A relatively large variation in the composition of the protein-free diets resulted in differences ($P<.05$) in the amino acid composition of endogenous protein recovered in ileal digesta in the present study (Table II.4). The amino acid composition of endogenous protein in the feces was relatively constant (Table II.5).

The true amino acid digestibilities were calculated in two diets to illustrate the effect of variation in amino acid composition of endogenous protein on true amino acid digestibilities (Table II.6). The apparent digestibilities of amino acids in the barley diet (103 g crude protein per kg dry matter) were derived from Sauer et al. (1981). The values for the barley-soybean meal diet (165 g crude protein per kg dry matter) were derived from Sauer and Thacker (1986). The ranges in true digestibilities were calculated using the ranges in recovery of amino acids in ileal digesta (Table II.2). The protein content of the barley diet was considerably lower than that of the

barley-soybean meal diet. This implies, assuming that the amount of endogenous protein in ileal digesta is relatively constant for both diets, that endogenous protein makes up a relatively larger proportion of total protein in ileal digesta when the barley as compared to the barley-soybean meal diet is fed. The true ileal amino acid digestibilities will therefore be higher for the barley than for the barley-soybean meal diet. For most amino acids the range in calculated true digestibilities was less than 2.5 percentage units (Table II.6). The variation in recovery of endogenous amino acids affected the calculated true digestibilities only to a small extent. The true ileal digestibilities of proline in both diets and of glycine in the barley diet exceeded 100% (Table II.6). The maximum true ileal digestibility can only be 100%, indicating that the endogenous levels of proline and glycine were overestimated.

The aforementioned method for calculating true protein and amino acid digestibilities has limitations. The feeding of protein-free diets change the metabolism of the animal and affect endogenous protein and amino acid losses (Krawielitzki et al., 1977; Low, 1980). In addition, the effect of the protein-containing diet itself on the endogenous protein and amino acid recoveries is not determined directly. Experiments with rats by Krawielitzki et al. (1977), using the ^{15}N-isotope dilution technique, showed that endogenous protein losses were positively related to the protein level in the diet. The results of the present experiment and Sauer et al. (1977) showed that feeding protein-free diets may overestimate endogenous proline and glycine recoveries as compared to feeding protein-containing diets. Apparently, the effect on endogenous losses varies for different amino

acids. A follow-up experiment, in which a protein-free diet is fed to pigs, while amino acids are administered intravenously, will elucidate the effect of protein status of the animal on endogenous amino acid recoveries and may provide more valid information on the amino acid composition of endogenous protein. A more valid estimation of the amino acid composition of endogenous protein is neccesary when the ^{15}N-isotope dilution technique is used to determine the endogenous amino acid recoveries.

E. BIBLIOGRAPHY

AOAC. 1980. Official Methods of Analyses (12th Ed.). Association of Official Analytical Chemists. Washington, DC.

Bergner, H. 1982. Fiber and nitrogen excretion. In: Physiology digestive chez le porc, Jouy-en-Josas. Les Colloques de l'INRA 12: 237.

Bergner, H., O. Simon and M. Zimmer. 1975. Einfluss des Gehaltes nativer Rohfaser in Diaeten von Ratten auf die Aminosaeuren-resorption. Arch. Tierernaehr. 25: 95.

Blackburn, S. 1968. Amino acid determination methods and techniques. Marcel Dekker Inc., New York.

Carlson, K. H. and H. S. Bayley. 1970. Nitrogen and amino acids in the feces of young pigs receiving a protein-free diet and diets containing graded levels of soybean meal or casein. J. Nutr. 100: 1353.

Fenton, T. W. and M. Fenton. 1979. An improved procedure for determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59: 631.

Gargallo, I. and D. R. Zimmerman. 1981. Effect of casein and starch infusion in the large intestine on nitrogen metabolism of growing swine. J. Nutr. 111: 1390.

Harvey, W. R. 1960. Least square analyses of data with unequal subclass numbers. USDA publ. ARS 20-8.

Holmes, J. H. G., H. S. Bayley and P. A. Leadbeater. 1974. Digestion of protein in small and large intestine of the pig. Brit. J. Nutr. 32: 479.

Horowitz, M. I. 1967. Section 6, Alimentary canal. In: C. F. Code (Ed.) Handbook of Physiology. American Physiological Society, Washington, D.C.

Kidder, D. E. and M. J. Manners. 1978. Digestion in the pig. Scientechnica, Bristol.

Krawielitzki, K., T. Volker, S. Smulikowska, H. D. Bock and J. Wuensche. 1977. Weitere Untersuchungen zum Multikompartment-Modell des Protein-stoffwechsels. Arch. Tierernaehr. 27: 609.

Low, A. G. 1980. Nutrient absorption in pigs. J. Sci. Food Agr. 31: 1087.

Low, A. G. 1982. Endogenous nitrogen evaluation from absorption studies In: Physiology digestive chez le porc, Jouy-en-Josas. Les Colloques de 1'INRA 12: 189.

Meinl, M. and F. Kreienbring. 1985. Untersuchungen zum Bakterienanteil im Schweinekot. Arch. Tierernaehr. 35: 33.

Mosenthin, R. 1987. Untersuchungen zum Einfluss pflanzlicher Kohlenhydrate in Rationen wachsender Schweine auf die endogene Stickstoff- und Enzymsekretion in den Verdauungstrakt sowie auf praecaecale und postileale Umsetzungen N-haltiger Verbindungen. Habilitationsschrift. Universitaet Kiel, F.R.G.

Mosenthin, R. and H. Henkel. 1983. Influence des substances vegetales de soutien sur le metabolisme azote du porc. In: Physiology digestive chez le porc, Jouy-en-Josas. Les Colloques de 1'INRA 16: 47.

NRC. 1979. Nutrient Requirements of Domestic Animals, No. 2. Nutrient requirements of Swine. Eighth Revised Ed. National Academy of

Sciences- National Research Council. Washington, D.C.

Ozimek, L., W. C. Sauer and G. Ozimek. 1985. The response of the secretion and activity of pancreatic enzymes to the quality and quantity of fat In: Proc. 3rd Intern. Seminar on Digestive Physiology in the Pig, Copenhagen: 146.

Sauer, W. C., H. Jorgensen and R. Berzins. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Can. J. Anim. Sci. 63: 233.

Sauer, W. C., J. J. Kennelly, F. X. Aherne and R.M. Cichon. 1981. Availabilities of amino acids in barley and wheat for growing pigs. Can. J. Anim. Sci. 61: 793.

Sauer, W. C. and L. Ozimek. 1986. Digestibility of amino acids in swine: results and their practical application. A review. Livestock Prod. Sci. 15: 367.

Sauer, W. C., S. C. Stothers and R. J. Parker. 1977. Apparent and true availabilities of amino acids in wheat and milling by-products for growing pigs. Can. J. Anim. Sci. 57: 775.

Sauer, W. C. and P. A. Thacker. 1986. Apparent ileal and fecal digestibility of amino acids in barley-based diets supplemented with soybean meal or canola meal for growing pigs. Anim. Feed Sci. Techn. 14: 183.

Schneeman, B. O., D. B. Richter and L. R. Jacobs. 1982. Response to dietary wheat bran in exocrine pancreas and intestine of rats. J. Nutr. 112: 283.

Souffrant, W. B., B. Darcy-Vrillon, T. Corring, J. P. Laplace, R. Koehler, G. Gebhardt and A. Rerat. 1986. Recycling of endogenous nitrogen in the pig. Arch. Tierernaehr. 36: 269.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of statistics. McGraw-Hill Book Co., New York.

Tanksley, F. D. and D. A. Knabe. 1984. Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. In: W. Haresign and D.J.A. Cole (Ed.) Recent Advances in Animal Nutrition. Butterworths, London: 75.

Taverner, M. R., I. D. Hume and D. J. Farell. 1981. Availability to pigs of amino acids in cereal grains 1: Endogenous levels of amino acids in ileal digesta and faeces of pigs given cereal diets. Brit. J. Nutr. 46: 149.

Wuensche, J., T. Zebrowska, U. Hennig, F. Kreienbring, M. Meinl, K. Krawielitzki, E. Borgmann, T. Voelker, B. Idzior, H.D. Bock and S. Buraczewski. 1979. Untersuchungen ueber die Proteinverdaulichkeit und Aminosaeurenresorption in verschiedenen Abschnitten des Verdauungstraktes beim Schwein. Arch. Tierernaehr. 29: 151.

Zebrowska, T. 1973. Digestion and absorption of nitrogenous compounds in the large intestine of pigs. Roczn Nauk Roln. 95B: 85.

Zebrowska, T. 1985. The influence of the level and source of fibre in the diet on the exocrine pancreatic secretion in growing pigs. In: Proc. 3rd Int. Seminar on Digestive Physiology in the Pig, Copenhagen: 152.

Table II.1. Formulation of the experimental diets.

Item	Control	Pectin	Cellulose	Fat
	---------- % ----------			
Ingredients				
Cornstarch	79.7	75.7	72.7	69.7
Alphafloc[a]	3.0	3.0	10.0	3.0
Sucrose	10.0	10.0	10.0	10.0
Canola oil	3.0	3.0	3.0	13.0
Pectin[b]	--	4.0	--	--
Iodized salt	.5	.5	.5	.5
Calcium carbonate	.5	.5	.5	.5
Dicalcium monophosphate	2.5	2.5	2.5	2.5
Vitamin mixture[c]	.2	.2	.2	.2
Mineral mixture[c]	.1	.1	.1	.1
Chromic oxide	.5	.5	.5	.5
Total	100.0	100.0	100.0	100.0

[a]Lee Chemicals Ltd, 1119 Yonge st., Toronto, Ontario, Canada, M4W 2L7

[b]Market Pantry, Bulk Speciality Food Ltd, 6655-178 st., Edmonton, Alberta, Canada, T5T 4J5

[c]The vitamin and mineral mixtures provided the following per kg diet: 1,300 IU vitamin A; 150 IU vitamin D3; 11 IU vitamin E; 2 mg vitamin K3; 2.2 mg riboflavin; 12 mg niacin; 11 mg pantothenic acid; 11 μg vitamin B12; 550 mg choline; 1.100 mg thiamine; 1.100 mg pyridoxine; .1 mg biotin; .6 mg folic acid; 50 mg Fe; 50 mg Zn; 2 mg Mn; 3 mg Cu; .14 mg I; .15 mg Se.

Table II.2. The recovery of endogenous protein and amino acids (g per kg dry matter intake), in addition to the digestibility of dry matter in ileal digesta in pigs fed different protein-free diets[a].

Item	Control(7)[b]	Pectin(7)	Cellulose(6)	Fat(6)	SE
Dry matter digestibility (%)	89.7[c]	86.2[d]	81.8[e]	87.3[d]	.4
Endogenous protein	19.8[d]	24.0[c]	22.5[cd]	20.0[d]	.9
Amino acids					
Indispensable					
Arginine	.73[cd]	.89[c]	.86[cd]	.67[d]	.05
Histidine	.22	.26	.23	.24	.01
Isoleucine	.36	.39	.37	.40	.02
Leucine	.60	.62	.62	.67	.03
Lysine	.53	.58	.56	.61	.03
Methionine	.16	.13	.17	.19	.01
Phenylalanine	.60	.63	.66	.64	.04
Threonine	.65	.69	.72	.75	.05
Valine	.48	.51	.50	.54	.03
Dispensable					
Alanine	.59	.68	.67	.64	.04
Aspartic acid	1.01	1.17	1.09	1.11	.05
Glutamic acid	1.16	1.31	1.23	1.32	.06
Glycine	1.94[d]	2.42[c]	2.33[c]	1.84[d]	.11
Proline	6.22[e]	8.43[c]	7.30[d]	5.74[e]	.34
Serine	.70	.80	.77	.76	.04
Tyrosine	.41	.43	.40	.43	.02

[a]Least square means and standard error of the means.
[b]Number of observations per treatment.
[c,d,e]Means in the same row with different letters differ ($P<.05$).

Table II.3. The recovery of endogenous protein and amino acids (g per dry matter intake), in addition to the digestibility of dry matter in feces in pigs fed different protein-free diets[a].

Item	Control	Pectin	Cellulose	Fat	SE
Dry matter digestibility (%)	94.3[b]	95.1[b]	89.2[c]	93.7[b]	.4
Endogenous protein	8.4[c]	9.0[c]	11.1[b]	8.5[c]	.5
Amino acids					
Indispensable					
Arginine	.32[c]	.35[c]	.43[b]	.34[bc]	.02
Histidine	.14[c]	.16[c]	.20[b]	.15[bc]	.01
Isoleucine	.35[c]	.37[c]	.47[b]	.36[c]	.02
Leucine	.53	.58	.70	.54	.04
Lysine	.51[c]	.56[c]	.71[b]	.53[c]	.03
Methionine	.24	.28	.31	.25	.02
Phenylalanine	.36	.41	.47	.39	.03
Threonine	.43[c]	.47[c]	.57[b]	.45[c]	.03
Valine	.40	.42	.52	.40	.03
Dispensable					
Alanine	.43[c]	.50[bc]	.61[b]	.47[c]	.03
Aspartic acid	.78[c]	.83[c]	1.06[b]	.79[c]	.05
Glutamic acid	.89[c]	.96[c]	1.19[b]	.92[c]	.06
Glycine	.37[c]	.41[c]	.50[b]	.39[bc]	.03
Proline	.26	.28	.35	.27	.02
Serine	.34[c]	.39[c]	.47[b]	.36[c]	.02
Tyrosine	.33	.37	.37	.34	.03

[a]Least square means and standard errors of the means.
[b,c]Means in the same row with different letters differ (P<.05).

Table II.4. The amino acid composition of endogenous protein (%) in addition to the recovery of endogenous protein (g per kg dry matter intake) in ileal digesta in pigs fed different protein-free diets[a].

Item	Control	Pectin	Cellulose	Fat	SE
Endogenous protein	19.8^{c}	24.0^{b}	22.5^{bc}	20.0^{c}	.9
Amino acids					
Indispensable					
Arginine	3.69^{b}	3.65^{b}	3.78^{b}	3.32^{c}	.09
Histidine	1.14^{bc}	1.10^{bc}	1.05^{c}	1.20^{b}	.03
Isoleucine	1.81^{c}	1.64^{c}	1.65^{c}	1.99^{b}	.05
Leucine	3.06^{bc}	2.63^{d}	2.79^{cd}	3.36^{b}	.10
Lysine	2.71^{c}	2.46^{c}	2.53^{c}	3.05^{b}	.10
Methionine	.81^{b}	.58^{c}	.80^{b}	.93^{b}	.06
Phenylalanine	3.08^{bc}	2.65^{c}	2.97^{bc}	3.21^{b}	.13
Threonine	3.27^{c}	2.92^{c}	3.22^{bc}	3.74^{b}	.13
Valine	2.42^{c}	2.14^{c}	2.22^{c}	2.71^{b}	.09
Dispensable					
Alanine	2.96^{bc}	2.84^{c}	2.94^{bc}	3.21^{b}	.09
Aspartic acid	5.14^{c}	4.92^{c}	4.88^{c}	5.57^{b}	.12
Glutamic acid	5.89^{c}	5.52^{c}	5.53^{c}	6.60^{b}	.10
Glycine	9.72^{c}	10.04^{bc}	10.30^{b}	9.22^{d}	.15
Proline	31.20^{c}	34.80^{b}	32.04^{c}	28.50^{d}	.84
Serine	3.56^{c}	3.37^{c}	3.45^{c}	3.85^{b}	.08
Tyrosine	2.13^{c}	1.80^{b}	1.80^{b}	2.19^{c}	.08

[a]Least square means and standard errors of the means.
[b,c,d]Means in the same row with different letters differ (P<.05).

Table II.5. The amino acid composition of endogenous protein (%) in addition to the recovery of endogenous protein (g per kg dry matter intake) in feces in pigs fed different protein-free diets[a].

Item	Control	Pectin	Cellulose	Fat	SE
Endogenous protein	8.4[c]	9.0[c]	11.1[b]	8.5[c]	.5
Amino acids					
Indispensable					
Arginine	3.83	3.85	3.84	3.97	.07
Histidine	1.73	1.75	1.76	1.78	.06
Isoleucine	4.15	4.14	4.27	4.12	.04
Leucine	6.45	6.25	6.41	6.10	.07
Lysine	6.11	6.12	6.39	6.16	.07
Methionine	2.90	3.07	2.76	2.90	.09
Phenylalanine	4.30[bc]	4.52[b]	4.17[c]	4.50[b]	.07
Threonine	5.15	5.20	5.20	5.30	.09
Valine	4.79	4.63	4.68	4.64	.10
Dispensable					
Alanine	5.23	5.56	5.48	5.46	.09
Aspartic acid	9.36	9.22	9.60	9.18	.11
Glutamic acid	10.61	10.63	10.73	10.68	.09
Glycine	4.45	4.51	4.46	4.50	.04
Proline	3.08	3.15	3.10	3.15	.08
Serine	4.08	4.28	4.19	4.21	.07
Tyrosine	3.88[b]	4.07[b]	3.31[c]	3.93[b]	.10

[a]Least square means and standard errors of means.
[b,c]Means in the same row with different letters differ ($P<.05$).

Table II.6. Apparent and true ileal protein and amino acid digestibilities in a barley and a barley-soybean meal diet.

	Apparent		True[a]	
	Barley[b]	Barley-SBM[c]	Barley	Barley-SBM
	%			
Protein	75.4	70.7	94.6 to 98.7	82.7 to 85.2
Amino acids				
Indispensable				
Arginine	79.2	81.1	91.2 to 95.1	88.1 to 90.4
Histidine	75.1	76.6	83.6 to 85.1	82.6 to 83.7
Isoleucine	76.3	77.0	84.3 to 85.2	82.4 to 83.0
Leucine	78.8	77.2	86.0 to 86.9	82.1 to 82.7
Lysine	72.5	75.1	85.1 to 87.0	81.7 to 82.6
Methionine	83.9	77.9	91.1 to 94.5	83.0 to 85.1
Phenylalanine	80.0	78.5	89.7 to 90.6	85.5 to 86.2
Threonine	67.4	67.7	84.1 to 86.6	78.1 to 79.7
Valine	69.4	73.7	77.3 to 78.3	78.8 to 79.5
Dispensable				
Alanine	68.4	66.9	81.0 to 82.9	75.0 to 76.2
Aspartic acid	69.0	70.6	84.1 to 86.5	78.1 to 79.3
Glutamic acid	85.4	84.2	89.4 to 89.9	87.5 to 87.9
Glycine	63.0	62.5	103.9 to 116.8	89.8 to 98.4
Proline	82.1	80.9	119.9 to 137.6	119.4 to129.6
Serine	74.2	72.5	88.2 to 90.2	81.6 to 82.9
Tyrosine	76.7	74.2	91.0 to 92.1	84.4 to 85.3

[a]Based on the range of recoveries of protein and amino acids in ileal digesta in the present study (Table II.2).
[b]Sauer et al. (1981), variety Klondike.
[c]Sauer and Thacker (1986).

III. THE EFFECT OF THE PROTEIN STATUS OF THE PIG ON THE RECOVERY AND AMINO ACID COMPOSITION OF ENDOGENOUS PROTEIN IN DIGESTA COLLECTED FROM THE DISTAL ILEUM[1]

A. INTRODUCTION

The ileal analysis method is the preferred method for the determination of amino acid digestibilities in feedstuffs for pigs at present (Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). Measurements obtained with this method are confounded by non-reabsorbed endogenous amino acids. With the traditional methods that are used to calculate true amino acid digestibilities it is assumed that the amount of endogenous protein, recovered in digesta collected from the distal ileum, and the amino acid composition of endogenous protein is not affected by the amount of protein or other components in the diet (Carlson and Bayley, 1970). Studies have indicated that these assumptions can be questioned (Krawielitzki et al., 1977; Souffrant et al., 1986; de Lange et al., 1988). The newly introduced ^{15}N-isotope dilution technique (Souffrant et al., 1986) allows for a clear differentiation between non-digested dietary and endogenous protein in the digestive tract, but the uncertainty remains that the amino acid composition is not affected by the concentration and type of dietary protein or the protein status of the animal.

A study was conducted to determine whether the intravenous infusion of a balanced amino acid mixture affected the recovery of

---

[1]A version of this chapter has been accepted for publication. de Lange, C. F. M., W. C. Sauer and W. B. Souffrant. 1988. J. Anim. Sci. (In press).

amino acids and the amino acid composition of endogenous protein in digesta collected from the distal ileum in pigs fed a protein-freediet, prior to the use of the ^{15}N-isotope dilution technique in ourlaboratory.

B. EXPERIMENTAL PROCEDURE

Four barrows (Lacombe x Yorkshire), with an average initial body weight of approximately 55 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to de Lange et al. (1988). Following surgery, the animals were housed individually in stainless steel metabolic crates in a temperature controlled barn (20 to 22°C). During the recovery period, with a duration of at least 10 d, the animals were fed an 18% crude protein starter diet (Sauer et al., 1983).

Polyvinylchloride catheters[2] (internal diameter 1.56 mm) were surgically implanted into the external jugular vein according to the method of Weirich et al. (1970), 2 d prior to the initiation of the first experimental period.

The experiment was conducted using a simple cross-over design. In each experimental period, the animals were offered 700 g of a protein-free diet (Table III.1), twice daily at 0800 and 2000 for a duration of 8 d. This diet was similar to the control diet used in a previous experiment (de Lange et al., 1988). Chromic oxide was included in the diet as the marker for the determination of the recovery of protein and amino acids in ileal digesta. Water was freely available from a low-pressure drinking nipple. A complete mixture of

---

[2]Argyle, Division of Sherwood Medical, St. Louis M. O. 63103 USA.

amino acids (Table III.2) was infused continuously into two animals via the jugular vein at a rate of 2,970 ml per d, using an Ismatic MP13 peristaltic pump[3]. A sterile saline solution (Table III.2) was infused in the other two animals at a similar rate and represented the control treatment. Ileal digesta were collected continuously for 24 h on d 8. The procedures involved in the collection were previously described by de Lange et al. (1988). The digesta were frozen immediately after collection.

The animals were allowed a recovery period of 8 d between the two experimental periods. During this time they were fed an 18% crude protein starter diet (Sauer et al., 1983). No blood could be withdrawn from the jugular catheters in two of the animals. It was, therefore, decided to remove the catheters and to place a new catheter in the opposite external jugular vein 2 d prior to the start of the second experimental period. However, problems with feed intake occurred and the experiment was repeated with animals of similar background and weight. Observations of animals, with a feed intake of less than 90% of the daily allowance, were considered invalid.

The animals were sacrificed at the conclusion of the experiment and dissected to determine whether cannulation had caused intestinal abnormalities.

Analytical and Statistical Procedures. Ileal digesta were pooled per animal and per experimental period after the conclusion of the experiment. The pooled samples were freeze-dried, ground in a Wiley mill through a 1.0-mm mesh screen and thoroughly mixed before samples

---

[3]Ismatic S.A., Limmastrasse 107/109, Zurich, Swiss.

were taken for further analyses. Analyses for nitrogen and dry matter were carried out according to AOAC (1980). Chromic oxide levels in feed and digesta were determined according to Fenton and Fenton (1979). Amino acid analyses were performed following acid hydrolysis in 6N HCl for 24 h using a Beckman 121MB amino acid analyzer[4] (Blackburn, 1968). All analyses were performed in duplicate.

The results were subjected to least square analyses of variance of unequal numbers (Harvey, 1960). Least square means for treatment differences were compared using the Student Newman-Keuls multiple range test (Steel and Torrie, 1980).

Four valid observations were obtained for each treatment from five different animals in three experimental periods. Cannulation did not result in intestinal abnormalities in these animals. A cross-over design could not be used for statistical analyses. When animals were used as blocks in a randomized block design, the animal effects were not significant ($P>.05$) for total endogenous protein (N x 6.25) and amino acid recoveries, as well as for amino acids expressed as a percentage of total endogenous protein. The animal effect was only significant ($P<.05$) for dry matter digestibility. When the period and treatment effects were included in the model as sources of variation, the period effect was not significant ($P>.05$) in all cases. It was therefore decided to use one way analyses of variance with treatment as source of variation to increase the degrees of freedom in the error term.

---

[4]Beckman Instruments Inc., Palo Alto, California 94304, USA.

C. RESULTS AND DISCUSSION

An average of 208 g of amino acids were administered parenterally per animal per day, which is below the NRC (1979) standards for total crude protein requirements for 35 to 60 kg growing pigs. The quality of the administered amino acid mixture, however, was extremely high. The daily allowance of lysine was 95% of the NRC (1979) standards. The daily allowance of the other amino acids exceeded NRC (1979) standards. The NRC (1979) values refer to total dietary intake of crude protein and amino acids, which are not completely available to the animal. Since the infused amino acids are readily available to the animal, the daily allowances of protein and amino acids most likely exceeded requirements.

The recoveries of endogenous protein and amino acids in ileal digesta are presented in Table III.3. These values refer to the balance of secretion and reabsorption, since a certain proportion of endogenous protein will be digested and reabsorbed (Low, 1982). The recovery of endogenous protein in ileal digesta was lower ($P<.05$) when amino acids were administered intravenously as compared to the infusion of saline. A major part of this difference was due to a decrease ($P<.05$) in the recovery of proline. The recoveries of the other amino acids were also reduced, although not significantly ($P>.05$).

The amount of endogenous protein recovered in ileal digesta for the control treatment (Table III.3) was lower than in the previous study (de Lange et al., 1988). This difference resulted largely from a lower recovery of proline. In fact, the recoveries of most of the other amino acids were higher in the present study. The proline recoveries in other studies ranged from 1.1 to 5.9 g per kg dry matter

intake (Holmes et al., 1974; Sauer et al., 1977; Wuensche et al., 1979; Taverner et al., 1981). The recovery of endogenous protein in the aforementioned studies ranged from 10.0 to 17.5 g per kg dry matter, which is in agreement with the present studies.

The recovery of proline in ileal digesta, when amino acids were administered intravenously (Table III.3), was lower than any of the values reported in the aforementioned studies. It can be speculated whether this reduction is due to a decreased secretion into the digestive tract or to an increased efficiency of reabsorption. Limited information is available on the exocrine secretory responses of gastro-intestinal organs to dietary protein levels. Corring and Saucier (1972) observed a reduction in pancreatic trypsin, chymotrypsin and amylase secretions when a protein-free diet was fed. There was no reduction in the total protein secretion. A reduction in the dietary protein content resulted in a decrease in the pancreatic secretions of trypsin, chymotrypsin, amylase and lipase as well as total protein in studies by Ozimek et al. (1985). Differences in pancreatic secretions of non-amino acid nitrogen may explain some of the differences between the observations of Corring and Saucier (1972) and Ozimek et al. (1985). Animals fed a protein-free diet will break down body protein, especially muscle protein, to supply amino acids for vital metabolic functions. Alanine and especially glutamine account for more than 50% of the total α-amino acid nitrogen released from muscle tissue (Rodwell, 1985). The tissue of the intestinal tract takes up large quantities of glutamine which can be metabolized to glutamate plus ammonia, citrulline and proline (Rodwell, 1985; Rogers and Phang, 1985). One can speculate that the relative large supply of glutamine

to the gut in pigs fed a protein-free diet may result in a higher rate of production of proline, which in turn can lead to an increased secretion of proline into the lumen of the gut. Karasov et al. (1987) showed that the absorptive capacity for amino acids in the small intestine in rats is affected by the protein content of the diet. The change in absorptive capacity per unit change in dietary protein content was largest for proline and aspartate and smallest for the indispensable amino acids, especially leucine. The capacity of the transport systems may be affected by the intravenous administration of amino acids and contribute to differences in the recovery of amino acids at the distal ileum.

When the recoveries of endogenous amino acids were expressed as a percentage of total endogenous protein, differences ($P<.05$) between treatments appeared for all amino acids with the exception of arginine, isoleucine, methionine, alanine, aspartic acid, glycine and serine (Table III.4). The high abundance of proline in ileal digesta from the control treatment depressed the concentration of the other amino acids. The concentrations were higher for all amino acids with the exception of arginine, alanine, glycine and proline than any of the values reported in other studies (Holmes et al., 1974; Sauer et al., 1977; Wuensche et al., 1979; Taverner et al., 1981; de Lange et al., 1988). These differences in amino acid composition of endogenous protein will affect the estimation of true amino acid digestibilities.

The true amino acid digestibilities in a barley and a barley-soybean meal diet were calculated (Tables III.5 and III.6). The apparent digestibilities of the barley diet (103 g crude protein per kg dry matter) were derived from Sauer et al. (1981). The values for the

barley-soybean meal diet (165 g crude protein per kg dry matter) were derived from Sauer and Thacker (1986). The first two columns of true digestibilities in Tables III.5 and III.6 were calculated using the recoveries of amino acids in ileal digesta determined in the present experiment (Table III.3). The values in columns 1 and 2 differed markedly for glycine and proline. Proline, and probably glycine recoveries in ileal digesta are most likely overestimated when protein-free diets are fed, resulting in calculated true amino acid digestibilities exceeding 100%. The differences for the important indispensable amino acids in practical diet formulation were relatively small. For the barley diet, these were 1.7, .5, and 2.3 percentage units for lysine, methionine, and threonine, respectively; for the barley-soybean meal diet these were .8, .3, and 1.5 percentage units, respectively. These differences were larger for the barley than for the barley-soybean meal diet. Because of the lower protein content of the barley diet, endogenous protein contributed relatively more to the total amount of protein recovered in ileal digesta. Differences in the recoveries of endogenous amino acids will result in larger differences between calculated true ileal amino acid digestibilities for the barley than for the barley-soybean meal diet. The protein status of the animal apparently does not affect the calculated true amino acid digestibilities of the important indispensable amino acids. The other criticism on the determination of true amino acid digestibilities remains, i.e., that endogenous protein and amino acid recoveries are not determined directly when protein-containing diets are fed.

The only available method, at present, to determine the endogenous protein recovery, when protein-containing diets are fed to

pigs, is the ^{15}N-isotope dilution technique (Souffrant et al., 1986). This technique, developed so far, only measures the total endogenous protein recovery for the determination of true protein digestibilities. Assumptions have to be made concerning the amino acid composition of endogenous protein in order to calculate true amino acid digestibilities from the true protein digestibilities determined with the ^{15}N-isotope dilution technique. The third column of true amino acid digestibilities (Tables III.5 and III.6) was calculated using the recovery of endogenous protein determined in the control treatment (Table III.3) and the amino acid composition of endogenous protein from animals in which amino acids were parenterally administered (Table III.4). The calculated true protein digestibilities are similar in columns 2 and 3 in Tables III.5 and III.6, respectively. The calculated true amino acid digestibilities differ, due to differences in the amino acid composition of endogenous protein (Table III.4). For the barley diet the differences were 4.4, 4.8, and 7.4 percentage units for lysine, methionine, and threonine, respectively; for the barley-soybean meal diet the differences were 2.4, 3.3, and 4.6 percentage units, respectively. These calculations illustrate that an accurate estimation of the amino acid composition of endogenous protein is critical when the true amino acid digestibilities are calculated, based on the determination of true protein digestibility with the ^{15}N-isotope dilution technique.

As was previously discussed by Sauer et al. (1977) and de Lange et al. (1988), the feeding of protein-free diets leads to an higer estimation of the recoveries of endogenous proline and glycine in ileal digesta as compared to when protein-containing diets are fed. On the

other hand, the total recovery of endogenous protein is probably underestimated when a protein-free diet is fed (Krawielitzki et al., 1977). The intravenous administration of a balanced amino acid mixture, as compared to saline, reduced the recovery of proline. The amino acid composition in ileal digesta determined in pigs fed a protein-free diet and parenterally administered with amino acids will probably provide a better estimation of the amino acid composition of pigs fed protein-containing diets than the composition in ileal digesta of pigs just fed a protein-free diet. Therefore, when the ^{15}N-isotope dilution technique is used to determine the true protein digestibility, then the amino acid composition in ileal digesta from pigs parenterally administered with amino acids should be used to calculate the true amino acid digestibilities.

D. BIBLIOGRAPHY

AOAC. 1980. Official Methods of Analyses (12th Ed.). Association of Official Analytical Chemists. Washington, DC.

Blackburn, S. 1968. Amino acid determination methods and techniques. Marcel Dekker Inc., New York.

Carlson, K. H. and H. S. Bayley. 1970. Nitrogen and amino acids in the feces of young pigs receiving a protein-free diet and diets containing graded levels of soybean meal or casein. J. Nutr. 100: 1353.

Corring, T. and R. Saucier. 1972. Sécrétion pancréatique sur proc fistulé. Adaption à la teneur en protéines du régime. Ann. Biol. anim. Bioch. Biophys. 12: 223.

de Lange, C. F. M., W. C. Sauer, R. Mosenthin and W. B. Souffrant. 1988. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. (In press), (This thesis, Chapter II).

Fenton, T. W. and M. Fenton. 1979. An improved procedure for determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59: 631.

Harvey, W. R. 1960. Least square analyses of date with unequal subclass numbers. USDA publ. ARS 20-8.

Holmes, J. H. G., H. S. Bayley and P. A. Leadbeater. 1974. Digestion of protein in small and large intestine of the pig. Brit. J. Nutr. 32: 479.

Karasov, W. H., D. H. Solberg and J. M. Diamond. 1987. Dependence of intestinal amino acid uptake on dietary protein or amino acid

levels. Am. J. Physiol. 252: G614.

Krawielitzki, K., T. Volker, S. Smulikowska, H. D. Bock and J. Wuensche. 1977. Weitere Untersuchungen zum Multikompartment-Modell des Protein- stoffwechsels. Arch. Tierernahr. 27: 609.

Low, A. G. 1982. Endogenous nitrogen evaluation from absorption studies In: Physiology digestive chez le porc, Jouy-en-Josas. Les Colloques de l'INRA 12: 189.

NRC. 1979. Nutrient Requirements of Domestic Animals, No. 2. Nutrient requirements of Swine. Eighth Revised Ed. National Academy of Sciences-National Research Council. Washington, D.C.

Ozimek, L., W. C. Sauer, G. Ozimek and D. M. Conway. 1985. Effect of diet on the qualitative and quantitative adaptation of exocrine pancreas secretions. Feeders day report, Dep. of Animal Science, Univ. of Alberta. 63: 16.

Rodwell, V. W. 1985. Catabolism of the carbon skeletons of amino acids. In: Harper's Review of Biochemistry. 20th Ed. Lange Medical Publications, Los Altos, California.

Rogers, Q. R. and J. M. Phang. 1985. Deficiency of pyrroline-5-carboxylate synthase in the intestinal mucosa of the cat. J. Nutr. 115: 146.

Sauer, W. C., H. Jorgensen and R. Berzins. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Can. J. Anim. Sci. 63: 231.

Sauer, W. C., J. J. Kenelly, F. X. Aherne and R.M. Cichon. 1981. Availabilities of amino acids in barley and wheat for growing pigs. Can. J. Anim. Sci. 61: 793.

Sauer, W. C. and L. Ozimek. 1986. Digestibility of amino acids in

swine: results and their practical application. A review. Livestock Prod. Sci. 15: 367.

Sauer, W. C., S. C. Stothers and R. J. Parker. 1977. Apparent and true availabilities of amino acids in wheat and milling by-products for growing pigs. Can. J. Anim. Sci. 57: 775.

Sauer, W. C. and P. A. Thacker. 1986. Apparent ileal and fecal digestibility of amino acids in barley-based diets supplemented with soybean meal or canola meal for growing pigs. Anim. Feed Sci. Techn. 14: 183.

Souffrant, W. B., B. Darcy-Vrillon, T. Corring, J. P. Laplace, R. Koehler, G. Gebhardt and A. Rerat. 1986. Recycling of endogenous nitrogen in the pig. Arch. Tierernahr. 36: 269.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of statistics. McGraw-Hill Book Co., New York.

Tanksley, F. D. and D. A. Knabe. 1984. Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. In: W. Haresign and D.J.A. Cole (Ed.) Recent Advances in Animal Nutrition. Butterworths, London: 75.

Taverner, M. R., I. D. Hume and D. J. Farell. 1981. Availability to pigs of amino acids in cereal grains 1: Endogenous levels of amino acids in ileal digesta and faeces of pigs given cereal diets. Brit. J. Nutr. 46: 149.

Weirich, W. E., J. A. Will and C. W. Crumpton. 1970. A technique for placing chronic indwelling catheters in swine. J. Appl. Physiol. 28: 117.

Wuensche, J., T. Zebrowska, U. Hennig, F. Kreienbring, M. Meinl, K. Krawielitzki, E. Borgmann, T. Voelker, B. Idzior, H.D. Bock and S.

Buraczewski. 1979. Untersuchungen ueber die Proteinverdaulichkeit und Aminosaeurenresorption in verschiedenen Abschnitten des Verdauungstraktes beim Schwein. Arch. Tierernahr. 29: 151.

Table III.1. Formulation of protein-free diet.

Ingredients	%
Cornstarch	79.7
Alphafloc[a]	3.0
Sucrose	10.0
Canola oil	3.0
Iodized salt	.5
Calcium carbonate	.5
Dicalcium phosphate	2.5
Vitamin mixture[b]	.2
Mineral mixture[b]	.1
Chromic oxide	.5
Total	100.0

[a]Lee Chemicals Ltd., 1119 Yonge St., Toronto, Ontario, Canada, M4W 2L7.

[b]The vitamin and mineral mixture provide the following per kg diet: 1,300 IU vitamin A; 150 IU vitamin D3; 11 IU vitamin E; 2 mg vitamin K3; 2.2 mg riboflavin; 12 mg niacin; 11 mg pantothenic acid; 11 μg vitamin B12; 550 mg choline; 1.1 mg thiamine; 1.1 mg pyridoxine; .1 mg biotin; .6 mg folic acid; 50 mg Fe; 50 mg Zn; 2 mg Mn; 3 mg Cu; .14 mg I; .15 mg Se.

Table III.2. Composition of the infusates.

Item	Vamin N[a]	Saline
Ingredients (per 100 ml)		
Amino acids:		
Indispensable:		
L-Arginine	330 mg	--
L-Histidine	240 mg	--
L-Isoleucine	390 mg	--
L-Leucine	530 mg	--
L-Lysine (as HCl)	390 mg	--
L-Methionine	190 mg	--
L-Phenylalanine	550 mg	--
L-Threonine	300 mg	--
L-Tryptophan	100 mg	--
L-Valine	425 mg	--
Dispensable:		
L-Alanine	300 mg	--
L-Aspartic acid	410 mg	--
L-Cysteine/L-Cystine (as HCl)	140 mg	--
L-Glutamic acid	900 mg	--
Glycine	210 mg	--
L-Proline	810 mg	--
L-Serine	750 mg	--
L-Tyrosine	50 mg	--
Electrolytes:		
Calcium ($CaCl_2$)	.5 mEq	.5 mEq
Chloride ion	5.5 mEq	5.5 mEq
Magnesium ($MgSO_4$)	.3 mEq	.3 mEq
Potassium (KCl, KOH)	2.0 mEq	2.0 mEq
Sodium (NaOH)	5.0 mEq	5.0 mEq

[a]Pharmacia Canada Inc., 2044 St.-Regis Blvd., Dorval, Quebec, Canada, H9P 1H6.

Table III.3. The recovery of endogenous protein and amino acids (g per kg dry matter intake), in addition to the digestibility of dry matter (%) at the distal ileum in pigs fed a protein-free diet and parenterally administered with amino acids or saline.

Item	Amino acids	Saline	SE[a]
Dry matter digestibility (%)	89.7	88.7	.8
Endogenous protein	12.7[c]	18.5[b]	1.6
Amino acids			
Indispensable:			
Arginine	.42	.62	.06
Histidine	.21	.26	.03
Isoleucine	.42	.47	.06
Leucine	.69	.77	.09
Lysine	.56	.63	.07
Methionine	.21	.22	.05
Phenylalanine	.74	.79	.10
Threonine	.82	.91	.10
Valine	.63	.65	.09
Dispensable			
Alanine	.59	.73	.09
Aspartic acid	1.02	1.24	.13
Glutamic acid	1.20	1.39	.15
Glycine	.83	1.44	.18
Proline	.61[c]	3.64[b]	.87
Serine	.66	.85	.09
Tyrosine	.41	.47	.05

[a]Standard error of least square means.
[b,c]Means in the same row with different letters differ (P<.05).

Table III.4. The amino acid composition of endogenous protein (%), in addition to the recovery of endogenous protein (g per kg dry matter intake) in ileal digesta in pigs fed a protein-free diet and parenterally administered with amino acids or saline.

Item	Amino acids	Saline	SE[a]
Endogenous protein	12.7[c]	18.5[b]	1.6
Amino acids (%)			
Indispensable:			
Arginine	3.29	3.36	.10
Histidine	1.66[b]	1.41[c]	.07
Isoleucine	3.28	2.52	.27
Leucine	5.46[b]	4.18[c]	.33
Lysine	4.41[b]	3.41[c]	.27
Methionine	1.63	1.20	.25
Phenylalanine	5.83[b]	4.28[c]	.40
Threonine	6.47[b]	4.97[c]	.37
Valine	4.97[b]	3.57[c]	.37
Dispensable:			
Alanine	4.62	3.96	.27
Aspartic acid	8.07	6.72	.43
Glutamic acid	9.39[b]	7.54[c]	.51
Glycine	6.64	7.75	.68
Proline	4.83[c]	19.35[b]	3.83
Serine	5.17	4.61	.31
Tyrosine	3.22[b]	2.56[c]	.19

[a]Standard error of least square means.
[b,c]Means in the same row with different letters differ (P<.05).

Table III.5. Apparent and true protein and amino acid digestibilities in a barley diet[a].

Item	Apparent	True[b]		
		1	2	3
Crude protein (%)	75.4	87.7	93.3	93.3
Amino acids (%)				
Indispensable:				
Arginine	79.2	86.7	90.3	90.1
Histidine	75.1	83.2	85.1	86.9
Isoleucine	76.3	85.6	86.7	89.9
Leucine	78.8	87.1	88.1	90.9
Lysine	72.5	85.8	87.5	91.9
Methionine	83.9	95.6	96.1	100.9
Phenylalanine	80.0	91.9	92.7	97.4
Threonine	67.4	88.4	90.7	98.1
Valine	69.4	79.7	80.1	84.5
Dispensable:				
Alanine	68.4	81.0	83.9	86.7
Aspartic acid	69.0	84.2	87.5	91.2
Glutamic acid	85.4	89.5	90.2	91.4
Glycine	63.0	81.4	95.0	89.9
Proline	82.1	86.1	106.1	88.0
Serine	74.2	87.4	91.2	93.4
Tyrosine	76.7	91.3	93.5	98.1

[a]Sauer et al. (1981), variety Klondike.
[b]Calculated in three different ways using:
1. recovery of protein and amino acids when administering amino acids parenterally (Table III.3)
2. recovery of protein and amino acids when administering saline parenterally (Table III.3)
3. recovery of protein when administering saline parenterally (Table III.3) and amino acid composition of endogenous protein when administering amino acids parenterally (Table III.4).

Table III.6. Apparent and true protein and amino acid digestibilities (%) in a barley-soybean meal diet[a].

Item	Apparent	True[b]		
		1	2	3
Crude protein	70.7	78.4	81.9	81.9
Amino acids				
Indispensable:				
Arginine	81.1	85.5	87.6	87.5
Histidine	76.6	82.3	83.6	84.9
Isoleucine	77.0	83.3	84.1	86.2
Leucine	77.2	82.9	83.5	85.5
Lysine	75.1	82.1	82.9	85.3
Methionine	77.9	86.0	86.3	89.6
Phenylalanine	78.5	87.1	87.7	91.1
Threonine	67.7	80.9	82.4	87.0
Valine	73.7	80.4	80.7	83.5
Dispensable:				
Alanine	66.9	75.0	76.9	78.7
Aspartic acid	70.6	78.2	79.8	81.6
Glutamic acid	84.2	87.6	88.1	89.1
Glycine	62.5	74.8	83.9	80.5
Proline	80.9	85.0	105.2	86.8
Serine	72.5	81.1	83.5	85.0
Tyrosine	74.2	84.7	86.2	89.5

[a]Sauer and Thacker (1986).
[b]Calculated in three different ways using:
1. recovery of protein and amino acids when administering amino acids parenterally (Table III.3)
2. recovery of protein and amino acids when administering saline parenterally (Table III.3)
3. recovery of protein when administering saline parenterally (Table III.3) and amino acid composition of endogenous protein when administering amino acids parenterally (Table III.4).

IV. REAL ILEAL PROTEIN AND AMINO ACID DIGESTIBILITIES IN FEEDSTUFFS FOR GROWING PIGS AS DETERMINED WITH THE ^{15}N-ISOTOPE DILUTION TECHNIQUE

A. INTRODUCTION

The ileal analysis method is the preferred method for the determination of amino acid digestibilities in feedstuffs for pigs at present (Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). Measurements obtained with this method are confounded with endogenous, amino acids and are therefore referred to as apparent digestibilities. Using conventional methods, the endogenous amino acid recoveries can not be quantified when protein-containing diets are fed (e.g. Carlson and Bayley, 1970). Apparent digestibilities corrected for endogenous protein or amino acid recoveries, as determined with the conventional methods, are referred to as true digestibilities. Using the ^{15}N-isotope dilution technique in which endogenous protein is labelled, including protein secreted into the gastro-intestinal lumen, a differentiation can be made between non-digested dietary and endogenous protein in the digestive tract (Souffrant et al., 1981, 1986). Apparent protein digestibilities corrected for endogenous protein recoveries, as determined by aid of the ^{15}N-isotope dilution technique, are referred to as real protein digestibilities. The ^{15}N-isotope dilution technique allows for the simultaneous study of factors that affect the real protein digestibilities and those that influence the endogenous protein recoveries. In addition, the real ileal amino acid digestibilities can be calculated from the real protein digestibilities obtained with the ^{15}N-isotope dilution

technique, if assumptions are made concerning the amino acid composition of endogenous protein recovered at the distal ileum (de Lange et al., 1988a,b). An experiment was conducted, using the ^{15}N-isotope dilution technique, to determine the endogenous protein recoveries at the distal ileum and to determine the real ileal protein and amino acid digestibilities when diets containing wheat, barley, canola meal, and soybean meal were fed to pigs.

B. EXPERIMENTAL PROCEDURE

Gilts, with an average initial body weight of 38 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The design of the cannulas were modified according to de Lange et al. (1988a). Following surgery, the animals were individually housed in stainless steel metabolic crates in a temperature controlled barn (20 to 22 °C). During recovery from surgery, the pigs were fed increasing amounts of the experimental diets (Table IV.1), until they consumed 700 g twice daily at 0800 and 2000. Water was freely available from a low-pressure drinking nipple. At least 7 d following the insertion of the cannulas, two polyvinylchloride catheters[1] (internal diameter 1.56 mm) were surgically implanted, one into each of the external jugular veins, according to procedures described by Weirich et al. (1970). The animals were allowed to recover from the second surgery for at least 3 d, or until they consumed 700 g of the experimental diet twice daily.

Four different feedstuffs were included in four experimental diets (Table IV.1). Diets 1 and 2 were cornstarch-based, formulated to

---

[1]Argyle, Division of Sherwood Medical. St. Louis M.O. 63103, USA.

contain approximately 16% crude protein (%Nx6.25) from soybean meal (SBM) or canola meal (CM), respectively. Dextrose was included to possibly improve the palatibility of these diets. Diets 3 and 4 contained 93.4% wheat and barley, respectively. All feedstuffs were ground through a one mm-mesh screen in a model D Comminutor Fitz-mill[2]. Canola oil was included in the diets to reduce dust formation. Vitamins and minerals were supplemented according NRC (1979) standards. Chromic oxide (.5%) was included in the diets to determine the nutrient digestibilities.

Following recovery, the animals were fed manually equal portions of the experimental diets every h for 3.5 d. The total daily feed allowance was maintained at 1400 g per d. A 9 d continuous intravenous infusion of ^{15}N-leucine (95% ^{15}N-enrichment), via one of the jugular catheters, was initiated 48 h after the start of hourly feeding. Approximately 40 mg ^{15}N-leucine, dissolved in a sterile saline solution, was infused per kg bodyweight per day. An Ismatic MP 13 peristaltic pump[3] was used to infuse the solution at a rate of approximately 575 ml per day. Blood and urine samples were taken every 2 h during the first 36 h of the infusion period while the pigs were fed hourly to study the rate of whole body protein turnover. Urine was collected by way of a Ruesch balloon catheter[4] (size 18) and a Citation 111-R2B closed urinary drainage system[5]. Following the 3.5 d hourly feeding period the pigs were fed twice daily 700 g of the experimental diets, at 0800 and 2000, for a period of 7.5 d. Blood and urine samples were taken every 12 h at feeding time. Throughout the 9

---

[2]Fitzpatrick Company, 832 Industrial Drive, Elmhurst, Ill 60126.
[3]Ismatic S.A., Limmastrasse 107/109, Zurich, Swiss.
[4]Ruesch, West Germany.
[5]Ingram and Bell, Don Mills, Ontario, Canada.

d infusion period, feces and urine were collected quantitatively to determine fecal and urinary ^{15}N excretion. Ileal digesta were collected continuously for 24 h on d seven and nine of the infusion period. The procedures involved in the collection of digesta were previously described by de Lange et al. (1988a).

Approximately 20 ml of blood were withdrawn from the catheter that was used for sampling. After sampling, the blood was immediately centrifuged at 3000 rotations per minute (rpm) in a Sorvall GLC-2B General Laboratory Centrifuge[6] for 10 min. Two 6 ml samples were taken from the supernatant (supern. 1) from each blood sample. The precipitate (prec. 1) was discarded. Ten percent trichloroacetic acid (TCA) was added to the supernatant, one to one on a volume basis, and the mixture was centrifugated at 3000 rpm for 10 min. The supernatant (supern. 2) and the precipitate (prec. 2) were separated and stored at 4 °C until further analyses. The excretion of urine was measured volumetrically every 2 or 12 h. The urine was acidified with five percent hydrochloric acid (HCl) to prevent ammonia losses and subsampled. The samples were stored at 4 °C until further analyses. Feces and ileal digesta were frozen immediately upon collection.

At the conclusion of the infusion period, the animals were sacrified to determine whether cannulation had caused intestinal abnormalities.

In addition to determining the real ileal protein digestibilities, it will be possible to relate the rate of whole body protein turnover and N retention to real ileal protein digestibilities with this experimental design. The results of the whole body protein

---

[6]Dupont company, Biomedical Division, Newton, Connecticut 06470.

turnover study will be reported at a later time.

Analytical and Statistical Procedures. After the conclusion of the experiment, feces and digesta were pooled per animal and collection day. The pooled samples were weighed, freeze-dried, weighed once more, ground in a model 4 laboratory Wiley mill[7] through a one mm-mesh screen, and thoroughly mixed before analyses. Analyses for N and dry matter content were carried out according to AOAC (1980) procedures. Amino acid analyses were performed following short-time pressure hydrolysis, using a Mikrotechna AAA 881 amino acid analyzer[8], according to the method described by Kreienbring and Wuensche (1976). The samples were pre-oxidized using performic acid for the analyses of methionine and cysteine (Kreienbring and Wuensche, 1976). Tryptophan was determined with a microbial assay using Lactobacillus plantarium (ATCC 8014) following alkaline hydrolyses (Kreienbring and Wuensche, 1976). Chromic oxide was determined according to Fenton and Fenton (1979). All analyses were performed in duplicate.

Prior to chemical analyses of blood plasma samples, the TCA precipitate (prec. 2) was resuspended in approximately 10 ml TCA and centrifugated at 3000 rpm for 10 min. The supernatant (supern. 3) was added to supern. 2 and considered to be the TCA-soluble fraction of blood and the precursor pool for the synthesis of endogenous protein secretions (Souffrant et al., 1981, 1986). TCA was added to a constant volume and then analyzed for N and ^{15}N-enrichment.

The distillate that remained after N analyses was quantitatively transferred to Kjeldahl bottles for ^{15}N-enrichment analyses in total N in feces, digesta and the TCA-soluble fraction of the blood.

---

[7]Arthur H. Thomas company, Philadelphia, P.A., USA.
[8]Mikrotechna, Prague, Czechoslovakia.

Thereafter 20 ml of 40% sodium hydroxide and zinc chips were added and the ammonia redistilled into a beaker containing 50 ml .01 normal HCl. The samples were re-distilled to separate the pH indicator, which interfered with the emission spectrometer, from the ammonia. The distillation was completed when 100 ml fluid were distilled into the beakers. The water was evaporated from the ammonium chloride solution in an oven at 60 °C. The remaining ammonium chloride was solubilized in 20 ml distilled water. This solution was introduced into a Isonitromat RFT 5201 emission spectrometer[9] for ^{15}N-enrichment analyses.

Ileal nutrient digestibilities were determined twice in each pig, using the 24 h pooled digesta samples, collected on d seven and nine of the infusion period, respectively. From the ratio ^{15}N-enrichment excess in total N in ileal digesta and the ^{15}N-enrichment excess in total N in the TCA-soluble fraction of the blood, the contribution of endogenous N to total N in ileal digesta can be calculated according to the following formula:

$$N_e = N_{dig} * \frac{E_f - E_{dig}}{E_f - E_{pl}}$$

N_e : endogenous N in digesta

N_{dig} : total N in digesta

E_f : percentage enrichment excess in feed

E_{dig} : percentage enrichment excess in digesta

E_{pl} : percentage enrichment excess in the TCA-soluble fraction of the blood.

---

[9]VEB Statron, Fuerstenwalde, G.D.R.

It was assumed that the ^{15}N-enrichment excess in the diets was 0. The real ileal protein digestibilities can then be calculated from the apparent ileal protein digestibilities and the recoveries of endogenous protein in ileal digesta as determined by aid of the ^{15}N-isotope dilution technique (Souffrant et al., 1981). The ^{15}N-enrichment excess in the endogenous protein secretions were assumed to be similar to the average ^{15}N-enrichment excess in the TCA-soluble fraction of the three blood samples that were taken during the collection of digesta. The real ileal amino acid digestibilities, in turn, can be calculated from the apparent amino acid digestibilities and the recovery of endogenous amino acids in ileal digesta. In these calculations, the amino acid composition of endogenous protein in ileal digesta was assumed to be similar to that of endogenous protein in ileal digesta of pigs fed a protein-free diet and simultanuously administered a well-balanced amino acid mixture parenterally as was previously reported by de Lange et al. (1988b). Amino acid analyses in the relevant samples of the studies by de Lange et al. (1988b) were repeated to conform to the same method of analyses that were carried out in the present study (Table IV.2). Valid observations were obtained from three animals per diet. Since problems with feed intake were initially observed, the start of hourly feeding and ^{15}N-leucine infusions were delayed in some instances. As a result, observations were not obtained in three experimental periods with four animals per period, as originally planned, and variation resulting from experimental period, therefore, could not be included in the statistical model. However, in a previous experiment it was shown that the experimental period had no effect on the recovery of endogenous

protein at the distal ileum (de Lange et al., 1988a). Two-way analyses of variation was used with diet and collection day as sources of variation (Harvey, 1960). Treatment means were compared using the Student Newman-Keuls multiple range test (Steel and Torrie, 1980).

C. RESULTS AND DISCUSSION

The proximate analyses and amino acid composition of the four diets are shown in Table IV.3. The amino acid composition of the protein-containing ingredients were similar to those reported in NRC (1988). The apparent protein and amino acid digestibilities (Table IV.4) were usually within the range of previously published ileal digestibilities in these feedstuffs (Sauer and Ozimek, 1986). The apparent protein and amino acid digestibilities of the CM diet were lower ($P<.05$) than in the SBM diet (Table IV.4). The differences in apparent digestibilities for protein, lysine and threonine were 17.8, 16.3 and 15.5 percentage units, respectively. The apparent digestibilities for protein and all of the amino acids, with the exception of lysine and threonine, were lower ($P<.05$) in barley than in wheat (Table IV.4). The difference in apparent protein digestibility between wheat and barley was 10.5 percentage units.

The apparent protein digestibilities are affected by the amount of endogenous protein recovered at the distal ileum. When true protein digestibilities are calculated in a conventional manner, it is assumed that the endogenous protein recoveries do not differ when different protein-containing diets are fed and that these endogenous recoveries can be estimated by feeding a protein-free diet (de Lange et al., 1988a,b). If the endogenous protein recoveries are assumed to be 12.7

g/kg dry matter intake, as was determined in a study in which pigs were fed a protein-free diet and simultaneously administered amino acids intravenously (de Lange et al, 1988b), the indirectly calculated true protein digestibilities were 90.7, 73.5, 88.8 and 80.8% for the SBM, CM, wheat and barley diets, respectively (Table IV.5). Similarly, the true amino acid digestibilities were calculated (Table IV.5).

A differentiation can be made between endogenous protein and non-digested dietary protein in ileal digesta or feces of pigs fed a protein-containing diet when the ^{15}N-isotope dilution technique is used (Souffrant et al., 1981, 1986). The real protein digestibilities in feedstuffs can thus be estimated directly. With this technique, endogenous rather than dietary protein is labelled. Labelled amino acids, that are administered orally, will be absorbed and reappear in the gastro-intestinal tract via pancreatic secretions within 6 h (Simon et al., 1983), complicating the differentiation between endogenous protein and non-digested dietary protein in the digestive tract. A basic requirement of the ^{15}N-isotope dilution technique is the establishment of steady-state conditions which can be achieved via a continuous intravenous infusion of ^{15}N-leucine. Due to transamination, the ^{15}N-label will also appear in other amino acids, so that not only the N in leucine is labelled (Matthews et al., 1979). The time course in the ^{15}N-enrichment excess in the several N-pools showed that after an initial period of rapid rise, the ^{15}N-enrichment excess in the different N-pools tended to plateau (Figure IV.1). During the last few days of the infusion period the ^{15}N-enrichment excess still rose slowly due to the recycling of labelled amino acids (Waterlow et al., 1978). In addition to infused ^{15}N, ^{15}N

originating from degraded body proteins will contribute to the ^{15}N-enrichment excess. For calculating the contribution of endogenous to total protein in ileal digesta, the TCA-soluble fraction of blood was considered to be the precurser pool for the synthesis of endogenous protein secreted into the gastro-intestinal lumen (Souffrant et al., 1981, 1986). The TCA-soluble fraction of blood contains free amino acids that are used for the synthesis of endogenous protein. Alpers (1972) demonstrated that amino acids, absorbed from the gastro-intestinal lumen, will also be used for protein synthesis in the intestinal wall without having to enter the main blood circulation. This implies that the ^{15}N-enrichment excess in amino acids, that are actually used for the synthesis of endogenous protein secretions, is probably lower than the excess measured in the TCA-soluble fraction of blood. As a result, the contribution of endogenous protein secretions to total protein in the gastro-intestinal tract is probably underestimated. Urea-N present in the TCA-soluble fraction of blood also contributes to the ^{15}N-enrichment excess in the TCA-soluble fraction. The ^{15}N-enrichment excess in urinary N was generally higher than that in the TCA-soluble fraction of blood (Figure IV.1). This implies that the ^{15}N-enrichment excess in urea was probably higher than in the free amino acids in the blood, which probably also resulted in an underestimation of the actual contribution of endogenous protein secretions to total protein in the gastro-intestinal tract.

The ^{15}N-enrichment excess in digesta, expressed as a percentage of the excess in the TCA-soluble fraction of blood, shows the relative contribution of endogenous protein secretions to total protein in ileal digesta. These relative contributions did not differ between d seven

and d nine of the infusion period (P>.10). Even though no real plateau was reached in the ^{15}N-enrichment excess in the different N-pools, no difference was observed in the relative contribution of endogenous protein secretions to total protein in ileal digesta between d seven and d nine of the infusion periods. The real protein digestibilities could therefore be calculated from these results.

The amount of endogenous protein in ileal digesta varied between 25.5 and 30.5 g/kg dry matter intake (Table IV.6). These values were much higher than those observed in a previous experiment in which pigs were fed a protein-free diet while amino acids were simultaneously administered intravenously (de Lange et al., 1988b) or the values observed in studies in which pigs were simply fed a protein-free diet (Wuensche et al, 1987; de Lange et al., 1988a). The quantities, observed in the present study, were also larger than those in studies in which alternative methods were used to determine the recovery of endogenous protein at the distal ileum in pigs: approximately 17.6 and 19.3 g per kg dry matter intake in pigs fed a barley-soybean meal diet (Zebrowska et al., 1982) and a fish meal-wheat diet (Simon et al., 1987), respectively. Zebrowska et al. (1982) calculated the contribution of endogenous to total protein in ileal digesta from the amino acid composition of dietary, endogenous and total protein in ileal digesta, assuming that the amino acid composition of endogenous protein was constant and that the true or real digestibilities were similar for all amino acids. The last assumption, in particular, can be questioned as feedstuffs contain a variety of different proteins. The amino acid composition and the real ileal digestibilities of these proteins may vary. Simon et al. (1987) used an alternative

^{15}N-isotope dilution technique in which protein containing diets were fed to pigs that were previously labelled with ^{15}N by feeding ^{15}N-ammonium carbonate and ^{15}N-ammonium chloride and in which the ^{15}N-enrichment excess in endogenous protein was assumed to be similar to that in urinary N. Since the ^{15}N-enrichment excess in urinary N was higher than that in the TCA-soluble fraction of the blood in the present study, it can be questioned whether the ^{15}N-enrichment excess in urinary N is a valid indicator for that in endogenous protein. Only when the microbes present in the gastro-intestinal tract preferentially incorporate N originating from urea into microbial protein and when the contribution of microbial protein to total protein in ileal digesta is larger than was previously estimated (Dierickx et al., 1986), than the ^{15}N-enrichment excess in urine may provide a better estimate of the enrichment excess in endogenous protein than that in the TCA-soluble fraction of blood. In addition, differences in diet composition may contribute to the observed differences in the recovery of endogenous protein at the distal ileum in the present study and those of Zebrowska et al. (1982) and Simon et al. (1987).

The present study showed that indirectly calculated true protein digestibilities underestimate the real protein digestibilities (Figure IV.2). The relatively low real protein digestibility in the CM diet (84.1 %, Table IV.7) is probably related to its high fiber content (Bell, 1984). Components such as lignin, pectins, tannins and cellulose in CM may interfere with the digestion of protein in this feedstuff (Bell, 1984). Furthermore, the real protein digestibility in the barley diet was lower than 100 % (94.2 %, Table IV.7). Eggum and Christensen (1975) showed that tannins, which are present in barley,

may bind to protein (amino acids) and as such affect digestion and (re-)absorption of both endogenous and exogenous protein.

Krawielitzki et al. (1977), in studies with rats, showed a linear relationship between the amount of dietary protein and the excretion of endogenous protein in feces, suggesting that the recovery of endogenous protein should be presented in units other than g/kg dry matter intake, especially if the protein content of the diets vary. Differences ($P<.05$) are apparent between diets if the recovery of endogenous protein is expressed as g per 100 g crude protein intake (Table IV.6). The recovery was highest for the barley (24.7 g per 100 g crude protein intake) and lowest for the SBM diet (13.7 g per 100 g protein intake). In theory, these results suggest, that, if the four feedstuffs would have been compared at a similar protein level, barley would have resulted in the largest endogenous protein recoveries. More studies are neccesary to identify the factors in these feedstuffs that are responsible for the observed differences in endogenous protein recoveries. The aforementioned considerations also illustrate that difficulties may arise in the interpretation of apparent protein digestibilities, especially when the protein content of the experimental diets vary and when different protein sources are included in a diet (Imbeah et al., 1988).

The ^{15}N-isotope dilution technique, developed so far, only measures the recovery of total endogenous protein in ileal digesta or feces, not the recovery of the individual endogenous amino acids. In order to calculate the real ileal amino acid digestibilities, the amino acid composition of endogenous protein is assumed to be similar to the amino acid composition of endogenous protein observed in a previous

experiment in which a protein-free diet was fed to pigs while amino acids were simultanuously administered intravenously (de Lange et al., 1988b), (Table IV.7). For some of the amino acids, especially in the wheat diet, the calculated real amino acid digestibilities exceeded 100 %. Since the amino acid content of some amino acids in wheat was very low (Table IV.3) and the real protein digestibility extremely high (Table IV.7), a small overestimation in the content of these amino acids in endogenous protein may result in overestimation of real amino acid digestibilities. Measuring the ^{15}N-enrichment excess in the individual amino acids as opposed to N in blood plasma and digesta may provide a better estimate of the real amino acid digestibilities. These results, however, indicate that the real amino acid digestibilities observed in the present study are higher than the true amino acid digestibilities determined in conventional digestibility studies (Tables IV.5 and IV.7).

In conclusion, the results of these studies, in which the ^{15}N-isotope dilution technique was used to determine the recovery of endogenous protein at the distal ileum in pigs, showed that the recoveries were much higher than previously determined in studies in which protein-free diets were fed. The differences in apparent protein digestibilities between the feedstuffs seemed to be more affected by the endogenous protein recoveries than by the differences in real protein digestibilities. The real amino acid digestibilities observed in the present study were also higher than the true amino acid digestibilities determined in studies in which protein-free diets were fed to determine the recovery of endogenous protein at the distal ileum in pigs.

D. BIBLIOGRAPHY

Alpers, D. H. 1972. Protein synthesis in intestinal mucosa: the effect of route of administration of precurser amino acids. J. of Clin. Invest. 51: 167.

AOAC. 1980. Official methods of analyses (12th Ed.). Association of Official Analytical Chemists. Washington, DC.

Bell, J. M. 1984. Nutrients and toxicants in rapeseed meal: a review. J. Anim. Sci. 58: 996.

Carlson, K. H. and H. S. Bayley. 1970. Nitrogen and amino acids in the feces of young pigs receiving a protein-free diet and diets containing graded levels of soybean meal or casein. J. Nutr. 100: 1353.

de Lange, C. F. M., W. C. Sauer, R. Mosenthin and W. B. Souffrant. 1988a. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. (In press), (This thesis, Chapter II).

de Lange, C. F. M., W. C. Sauer and W. B. Souffrant. 1988b. The effect of the protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. J. Anim. Sci. (In press), (This thesis, Chapter III).

Dierickx, N. A., I. J. Vervaeke, J. A. Decuypere and H. K. Henderickx. 1986. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs II. studies in vivo. Livestock Prod. Sci. 14: 177.

Eggum, B. O. and K. D. Christenson. 1975. Influence of tannin on protein utilization of feedstuffs with special reference to

barley. In: Breeding for seed improvement using nuclear Techniques. International Atomic Energy Agency. Vienna: 135.

Fenton, T. W. and M. Fenton. 1979. An improved procedure for determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59: 631.

Harvey, W. R. 1960. Least square analyses of data with unequal subclass numbers. USDA publ. ARS 20-8.

Imbeah. M., W. C. Sauer and R. Mosenthin. 1988. The prediction of the digestible amino acid supply in barley-soybean meal or canola meal diets and pancreatic secretions in pigs. J. Anim. Sci. 66: 1409.

Krawielitzki, K., T. Volker, S. Smulikowska, H. D. Bock and J. Wuensche. 1977. Weitere Untersuchungen zum Multikompartment-Modell des Protein-Stoffwechsels. Arch. Tierernaehr. 27: 609.

Kreienbring F. and J. Wuensche. 1976. Darlegung anwendbare Methoden zur Proteinhydrolyse und Aminosauerenbestimmung. Tag. Ber. Akad. Landwirtsch. Wiss. G. D. R., Berlin 142: 27.

Matthews, D. E., E. B. Ben-Galim and D. M. Bier. 1979. Determination of stable isotopic enrichment in individual plasma amino acids by chemical ionization-mass spectrometry. Anal. Chem. 51: 80.

NRC. 1979. Nutrient requirements of domestic animals, No. 2. Nutrient requirements of swine. Eigth Revised Ed. National Academy of Sciences- National Research Council. Washington, D.C.

NRC. 1988. Nutrient requirements of domestic animals, No. 2. Nutrient requirements of swine. Ninth Revised Ed. National Academy of Sciences- National Research Council. Washington, D.C.

Sauer, W. C., H. Jorgensen and R. Berzins. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in

feedstuffs for pigs. Can. J. Anim. Sci. 63: 233.

Sauer, W. C. and L. Ozimek. 1986. Digestibility of amino acids in swine: results and their practical application. A review. Livestock Prod. Sci. 15: 367.

Simon, O., T. Zebrowska, H. Bergner and R. Muenchmeyer. 1983. Investigations on the pancreatic and stomach secretions in pigs by means of continuous infusion of ^{14}C amino acids. Arch. Tierernaehr. 33: 9.

Simon, O., H. Bergner and I. G. Partridge. 1987. Estimation of endogenous N proportions in ileal digesta and faeces in ^{15}N-labelled pigs. Arch. Tierernaehr. 10: 851.

Souffrant, W. B., R. Koehler, R. Matkowitz, G. Gebhardt and H. Schmandke. 1981. Ernaehrungsphysiologische Untersuchungen an Schweinen zur beurteilung von modifizierten Proteinen. Arch. Tierernaehr. 31: 675.

Souffrant, W. B., B. Darcy-Vrillon, T. Corring, J. P. Laplace, R. Koehler, G. Gebhardt and A. Rerat. 1986. Recycling of endogenous nitrogen in the pig. Arch. Tierernaehr. 36: 269.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of statistics. McGraw-Hill Book Co., New York.

Tanksley, F. D. and D. A. Knabe. 1984. Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. In: W. Haresign and D. J. A. Cole (Ed.) Recent Advances in Animal Nutrition. Butterworths, London: 75.

Waterlow, J. C., P. J. Garlick and D. J. Millward. 1978. Protein turnover in mammalian tissues and in the whole body. North Holland, Amsterdam.

Weirich, W. E., J. A. Will and C. W. Crumpton. 1970. A technique for placing chronic indwelling catheters in swine. J. Appl. Physiol. 28: 117.

Wuensche, J., U. Hermann, M. Meinl, U. Hennig, F. Kreienbring and P. Zwierz. 1987. Einfluss exogener Faktoren auf die praezaekale Naehrstoff und Aminosaeurenresorption ermittelt an Schweinen mit Ileo-Rektal-Anastomosen. Arch. Tierernaehr. 37: 745.

Zebrowska, T., O. Simon, R. Muenchmeyer, E. Wolf, H. Bergner and H. Zebrowska. 1982. Flow of endogenous and exogenous amino acids along the gut of pigs. Arch. Tierernaehr. 32: 431.

Table IV.1. Formulation of the experimental diets.

Item	Soybean meal	Canola meal	Wheat	Barley
Ingredients (%)				
Soybean meal	35.5			
Canola meal		44.5		
Wheat			93.9	
Barley				93.9
Cornstarch	48.3	44.0		
Sucrose	10.0	10.0		
Canola oil	3.0	3.0	3.0	3.0
Iodized salt	.5	.5	.5	.5
Calcium carbonate	.8	.7	1.1	1.1
Dicalcium monophosphate	1.1		.7	.7
Vitamin mixture[a]	.2	.2	.2	.2
Mineral mixture[a]	.1	.1	.1	.1
Chromic oxide	.5	.5	.5	.5
Total	100.0	100.0	100.0	100.0

[a]The vitamin and mineral mixture provided the following per kg diet: 1,300 IU vitamin A; 150 IU vitamin D3; 11 IU vitamin E; 2 mg vitamin K3; 2.2 mg riboflavin; 12 mg niacin; 11 mg pantothenic acid; 11 μg vitamin B12; 550 mg choline; 1 1 mg thiamine; 1.1 mg pyridoxine; .1 mg biotin; .6 mg folic acid; 50 mg Fe; 50 mg Zn; 2 mg Mn; 3 mg Cu; .14 mg I; .15 mg Se.

Table IV.2. Amino acid composition of endogenous protein[a].

Amino acids	(g/16 g N)
Indispensable:	
Arginine	3.11
Histidine	1.19
Isoleucine	3.35
Leucine	5.23
Lysine	4.01
Methionine	
Phenylalanine	3.56
Threonine	6.49
Tryptophan	
Valine	4.68
Dispensable:	
Alanine	5.15
Aspartic acid	8.54
Cysteine	
Glutamic acid	9.88
Glycine	7.12
Proline	5.74
Serine	5.27
Tyrosine	3.92

[a]According to de Lange et al (1988b) and re-analyzed according to Kreienbring and Wuensche (1976).

Table IV.3. Proximate analyses and amino acid content of the experimental diets.

Item	Soybean meal	Canola meal	Wheat	Barley
Proximate analyses (%)[a]				
Dry matter	89.6	90.5	87.3	88.2
Crude protein	18.6	16.9	14.4	11.2
Ether extract	4.1	5.1	5.3	5.3
Crude fiber	1.5	6.2	2.6	3.7
Nitrogen-free extract	70.7	67.1	73.4	75.2
Amino acids (g/16 g N):				
Indispensable:				
Arginine	6.77	5.62	4.54	4.58
Histidine	2.33	2.31	2.02	1.85
Isoleucine	4.74	4.46	3.99	4.15
Leucine	7.97	7.69	7.30	7.68
Lysine	5.94	5.58	2.52	3.55
Methionine	1.47	1.92	1.67	1.63
Phenylalanine	4.83	4.13	4.70	5.20
Threonine	3.44	4.25	2.37	3.37
Tryptophan	1.27	1.16	1.17	.99
Valine	4.29	4.93	4.20	4.95
Dispensable:				
Alanine	4.30	4.54	3.61	4.09
Aspartic acid	11.40	7.80	5.11	6.00
Cysteine	1.67	2.48	2.48	2.54
Glutamic acid	18.62	17.91	30.69	23.93
Glycine	4.26	5.28	4.18	4.03
Proline	4.86	6.40	10.26	11.81
Serine	5.64	4.93	5.11	4.35
Tyrosine	2.97	2.54	2.45	2.71

[a]Dry matter basis.

Table IV.4. Apparent ileal protein, dry matter and amino acid digestibilities (%) in the experimental diets.

	Soybean meal	Canola meal	Wheat	Barley	SE[a]
Protein	83.8[b]	66.0[c]	80.0[b]	69.5[c]	1.6
Dry matter	81.7[b]	66.4[e]	75.2[c]	70.4[d]	.6
Amino acids					
Indispensable:					
Arginine	91.9[b]	80.3[cd]	84.0[c]	76.5[d]	1.7
Histidine	87.7[b]	77.4[d]	83.4[c]	74.8[d]	1.0
Isoleucine	85.7[b]	69.4[d]	83.5[b]	75.0[c]	1.0
Leucine	85.6[b]	72.3[d]	85.1[b]	77.5[c]	.9
Lysine	87.3[b]	71.0[c]	71.4[c]	69.8[c]	1.8
Methionine	87.6[b]	79.8[c]	87.6[b]	78.0[c]	1.2
Phenylalanine	82.0[b]	68.4[d]	85.9[b]	75.6[c]	1.8
Threonine	75.2[b]	59.7[c]	66.0[c]	65.6[c]	1.9
Tryptophan	83.5[b]	63.1[c]	79.9[b]	59.2[c]	3.0
Valine	81.9[b]	67.0[d]	81.7[b]	76.7[c]	1.0
Dispensable:					
Alanine	81.4[b]	71.8[c]	75.5[c]	65.3[d]	1.5
Aspartic acid	85.5[b]	65.6[d]	74.4[c]	67.3[d]	1.4
Cysteine	74.5[c]	62.8[d]	81.9[b]	74.7[c]	1.3
Glutamic acid	87.2[c]	81.1[d]	94.2[b]	87.1[c]	.7
Glycine	74.4[b]	60.7[c]	61.8[c]	43.2[d]	3.5
Proline	80.2[b]	61.7[bc]	73.0[b]	48.0[c]	6.9
Serine	87.4[b]	73.0[c]	85.4[b]	75.1[c]	1.3
Tyrosine	86.3[b]	71.9[d]	86.0[b]	79.1[c]	1.4

[a]Standard error of the means.
[b,c,d,e]Values in the same row followed by different superscripts differ significantly ($P<.05$).

Table IV.5. True ileal protein and amino acid digestibilities[a] (%) in the experimental diets.

	Soybean meal	Canola meal	Wheat	Barley	SE[b]
Protein	90.7[c]	73.5[e]	88.8[c]	80.8[b]	1.6
Amino acids					
Indispensable:					
Arginine	95.1[c]	84.4[e]	90.1[d]	84.2[e]	1.7
Histidine	91.1[c]	81.3[d]	88.6[c]	82.1[d]	1.0
Isoleucine	90.6[c]	75.0[e]	90.9[c]	84.1[d]	1.0
Leucine	90.0[c]	77.4[e]	91.4[c]	85.2[d]	0.9
Lysine	92.0[c]	76.4[e]	85.4[d]	82.6[d]	1.8
Methionine					
Phenylalanine	87.1[d]	74.9[e]	92.6[c]	83.4[d]	1.8
Tryptophan					
Threonine	88.0[c]	71.1[d]	90.2[c]	87.5[c]	1.9
Valine	89.3[cd]	74.1[e]	91.6[c]	87.4[d]	1.0
Dispensable:					
Alanine	89.5[c]	80.3[d]	88.1[c]	79.6[d]	1.5
Aspartic acid	90.6[c]	73.8[e]	89.1[c]	83.5[d]	1.4
Cysteine					
Glutamic acid	90.8[d]	85.2[e]	97.0[c]	91.8[d]	.7
Glycine	85.8[c]	70.8[de]	76.9[cd]	63.2[e]	3.5
Proline	88.3[c]	68.4[c]	77.9[c]	53.5[d]	6.9
Serine	93.8[c]	81.1[e]	94.6[c]	88.8[d]	1.3
Tyrosine	95.3[cd]	83.4[e]	100.1[c]	95.5[d]	1.4

[a]Indirectly calculated, assuming 12.7 g endogenous protein per kg dry matter intake and assuming the amino acid composition of endogenous protein to be similar to that observed in pigs fed a protein-free diet and simultanuously administered with amino acids parenterally (de Lange et al., 1988b)

[b]standard error of the means.

[c,d,e]Values in the same row followed by different superscripts differ significantly ($P<.05$).

Table IV.6. Endogenous protein in ileal digesta of pigs fed protein-containing diets as determined with the ^{15}N-isotope dilution technique.

	Soybean meal	Canola meal	Wheat	Barley	SE[a]
g per kg DM intake	25.5	30.5	27.4	27.7	2.4
percent of total CP present at the distal ileum	84.6[c]	53.5[d]	94.5[b]	81.1[c]	3.0
g per 100 g CP intake	13.7[c]	18.0[c]	19.1[c]	24.7[b]	1.7

[a]standard error of the means.
[b,c,d]values in the same row followed by different superscripts differ significantly ($P<.05$).

Table IV.7. Real ileal protein and amino acid digestibilities[a] (%) in the experimental diets.

	Soybean meal	Canola meal	Wheat	Barley	SE[b]
Protein	97.5[c]	84.1[e]	99.0[c]	94.2[d]	1.0
Amino acids					
Indispensable:					
Arginine	98.2[c]	90.2[d]	97.1[c]	93.3[d]	1.1
Histidine	94.6[c]	86.7[e]	94.7[c]	90.7[d]	.9
Isoleucine	95.4[d]	82.9[e]	99.5[c]	95.0[d]	1.2
Leucine	94.5[d]	84.5[e]	98.8[c]	94.3[d]	1.1
Lysine	96.6[d]	84.0[e]	101.7[c]	97.7[d]	1.2
Methionine					
Phenylalanine	92.1[d]	83.9[e]	100.3[c]	92.5[d]	1.8
Threonine	101.0[d]	87.2[e]	118.2[c]	113.3[c]	3.3
Tryptophan					
Valine	96.8[d]	84.1[e]	103.0[c]	100.1[cd]	1.7
Dispensable:					
Alanine	97.8[d]	92.3[e]	102.7[c]	96.5[de]	1.4
Aspartic acid	95.7[d]	85.3[e]	106.2[c]	102.6[c]	1.8
Cysteine					
Glutamic acid	94.5[e]	91.0[f]	100.3[c]	97.4[d]	.8
Glycine	97.3[c]	85.0[d]	94.3[c]	86.9[d]	2.4
Proline	96.4[c]	77.9[cd]	83.7[c]	60.0[d]	6.3
Serine	100.2[d]	92.3[e]	105.1[c]	104.9[c]	1.3
Tyrosine	104.4[d]	99.7[d]	116.5[c]	114.9[c]	2.4

[a]Directly determined real protein digestibilities using the ^{15}N-isotope dilution technique. The real amino acid digestibilities are estimated from the apparent protein and amino acid digestibilities, the real protein digestibilities and the amino acid composition of endogenous protein as observed in pigs fed a protein-free diet and simultanuously administered with amino acids parenterally (de Lange et al., 1988b).

[b]Standard error of the means.

[c,d,e,f]Values in the same row followed by different superscripts differ significantly ($P<.05$).

Figure IV.1. Time course of ^{15}N-enrichment excess in urine, digesta and the TCA-soluble fraction of blood in a pig fed a soybean meal diet and continuously administered ^{15}N-leucine intravenously.

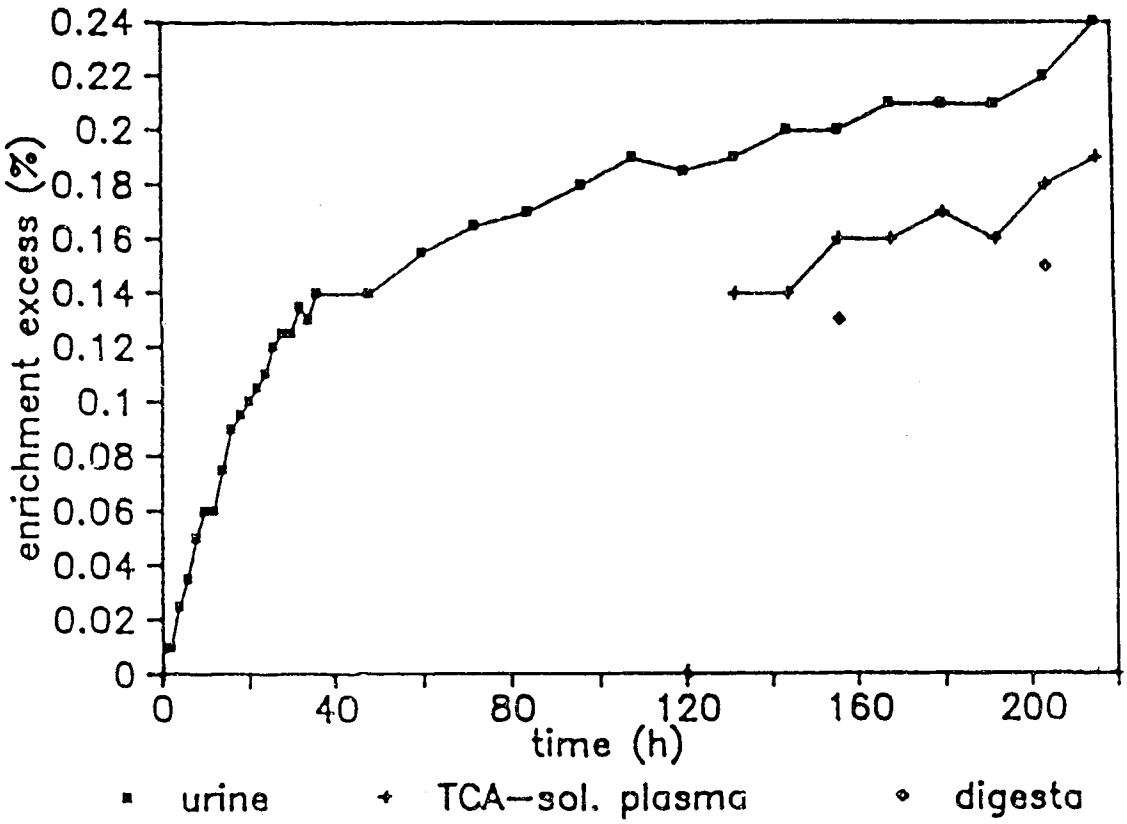
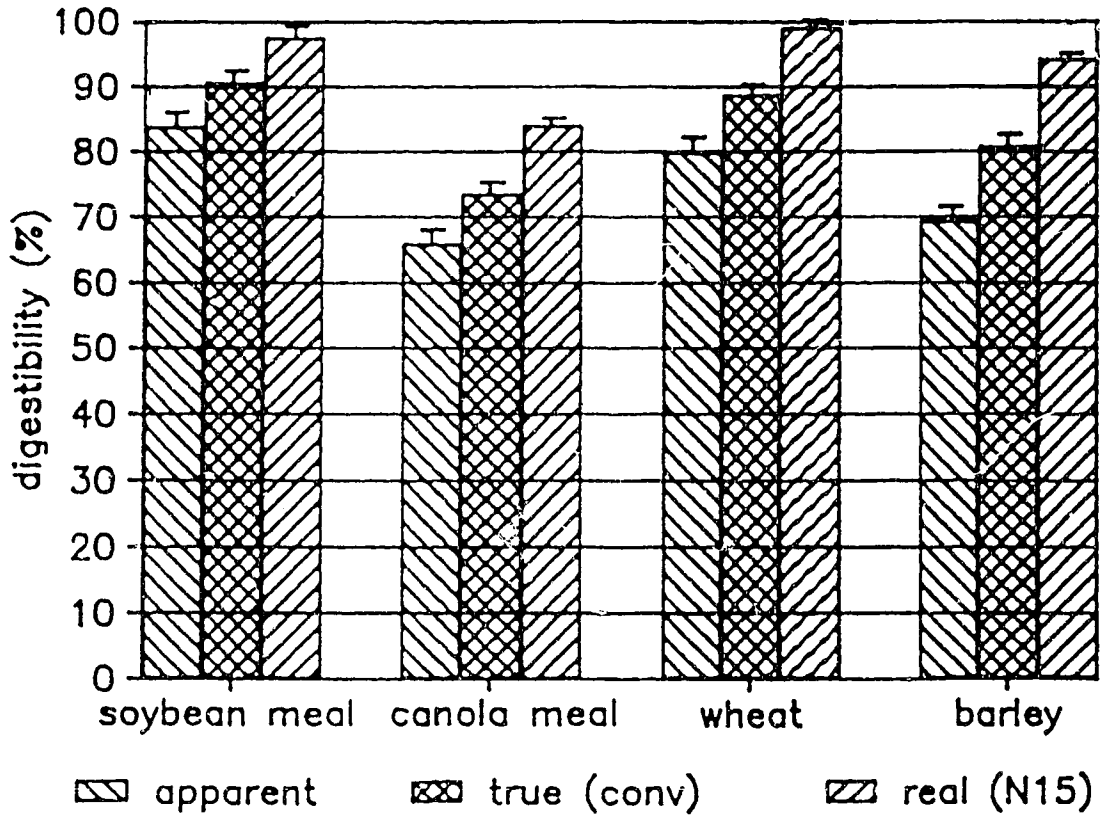


Figure IV.2. Apparent, true and real ileal protein digestibilities in the experimental diets.



V. ^{15}N-LEUCINE AND ^{15}N-ISOLEUCINE ISOTOPE DILUTION TECHNIQUES VERSUS THE ^{15}N-ISOTOPE DILUTION TECHNIQUE FOR DETERMINING THE RECOVERY OF ENDOGENOUS PROTEIN AND AMINO ACIDS IN DIGESTA COLLECTED FROM THE DISTAL ILEUM IN PIGS

A. INTRODUCTION

The ^{15}N-isotope dilution technique appears to be one of the most valid methods for determining the recovery of endogenous protein at the distal ileum and in the feces of pigs fed protein-containing diets (Souffrant et al., 1981, 1986; Low, 1982; Sauer and Ozimek, 1986; de Lange et al., 1988a,b,c). With this technique, ^{15}N-labelled amino acids, usually in the form of ^{15}N-labelled leucine, are infused intravenously for up to nine days to label endogenous protein. By estimating the ^{15}N-enrichment excess in endogenous protein and feces or digesta, the contribution of endogenous to total protein in the digestive tract can be determined. The trichloroacetic acid (TCA)-soluble fraction of the blood is considered to be the precursor pool for endogenous protein synthesis in the ^{15}N-isotope dilution technique; the enrichment excess in the total N in this fraction of blood is considered to be similar to that in endogenous protein secreted into the digestive tract (Souffrant et al., 1981, 1986; de Lange et al., 1988c)

The ^{15}N-enrichment excess is not similar for all amino acids and other N-containing molecules, such as urea, that are present in the TCA-soluble fraction of the blood when ^{15}N-leucine is infused continuously (Matthews et al., 1979). A source of error will be introduced when the ^{15}N-isotope dilution technique is used for determining the recovery of endogenous protein in ileal digesta if the

relative contribution of amino acids, or urea, to the enrichment excess in the total N in the TCA-soluble fraction of blood is different from their contribution to the enrichment excess in endogenous protein recovered at the distal ileum.

Gas chromatography-mass spectrometry (GCMS) enables measurement of ^{15}N-enrichment excess in single plasma amino acids (Matthews et al., 1979; Krishnamurti and Schaefer, 1987). Using single ion monitoring-GCMS, the ^{15}N-enrichment excess in leucine and isoleucine was measured in blood plasma and digesta of pigs into which ^{15}N-leucine was infused continuously (de Lange et al., 1988c). The objectives of these studies were to determine by aid of alternative ^{15}N-leucine and ^{15}N-isoleucine isotope dilution techniques the contributions of endogenous leucine, isoleucine and protein to total leucine, isoleucine and protein in digesta, respectively, and to compare these results to those obtained with the ^{15}N-isotope dilution technique.

B. EXPERIMENTAL PROCEDURE

A detailed description of the experimental procedures were previously presented (de Lange et al., 1988c). In summary, twelve gilts, fitted with a simple T-cannula at the distal ileum and two catheters in the external jugular veins, were fed one of four experimental diets. ^{15}N-leucine was infused continuously for 9 d at a rate of approximately 40 mg per kg bodyweight per d via one of the jugular catheters. Blood samples were taken twice daily during feeding time. Digesta were collected continuously for 24 h on d seven and nine of the infusion period.

Samples of blood and digesta were taken and prepared for analyses as

previously described (de Lange et al., 1988c). Immediately after the blood samples were taken, they were separated into a trichloroacetic acid (TCA)-soluble fraction and a TCA-precipitable fraction of blood plasma, which contains the plasma proteins. The TCA-soluble fraction of blood plasma samples (6 ml), which contains free plasma amino acids and other small plasma molecules such as urea, was diluted to 25 ml with ten percent TCA. Samples were taken from this solution for analyses of ^{15}N-enrichment excess in total nitrogen, leucine and isoleucine.

Analytical Procedure. The methods for analyses of dry matter, chromic oxide, crude protein (Nx6.25) and amino acids in feed and digesta were previously described (de Lange et al., 1988c). The ^{15}N-enrichment excess in the TCA-soluble fraction of blood was determined using an Isonitromat RFT 5201 emission spectrometer (de Lange et al., 1988c).

^{15}N-enrichment excess in leucine and isoleucine was determined according to procedures adapted from Haymond et al. (1980) and Krishnamurti and Schaefer (1987). In this procedure, negatively charged ions and neutral organic compounds are removed from the samples using anion and cation exchange columns. Thereafter, the samples are freeze-dried and trimethylsilyl (TMS) derivatives prepared with addition of N,O-bis-trimethylsilyl-trifluoracetamide (BSTFA)[1] and acetonitrile[1]. The samples are then injected into the gas chromatograph-mass spectrometer (GCMS).

TCA had to be removed from the samples prior to analyses as it will be derivatized by BSTFA,. 800 μl of the 25 ml samples, containing the TCA-soluble fraction of the blood, were mixed with an equal volume of

---

[1]Sigma Chemical Company, P.O. Box 14508, St. Louis, M.O. 63178

diethyl ether. The ether, used to extract TCA, was removed and discarded. This procedure was repeated three times. The remaining solution (pH 2-3) was applied to two ml AG1-X8 anion-exchange resin[2] (200-400 mesh, formate form) placed in three ml syringes from which the stopper was removed. The effluent was directly applied to two ml AG 50-X8 cation-exchange resin[2] (200-400 mesh, hydrogen form), also placed in three ml syringes. The anion- and cation-exchange resins were washed previously with neutralized deionized water. After the samples were applied to the anion-exchange column, the columns were washed five times with one ml portions of neutral deionized water. The anion-exchange column was then discarded and the cation-exchange column washed twice times with one ml neutral deionized water. The effluent of the cation-exchange column was discarded. The amino acids were eluded from the cation-exchange column with freshly prepared 4 N NH_4OH solution. Firstly, 1 ml of the NH_4OH solution was applied to the column and the effluent discarded. Thereafter, 4 ml of the NH_4OH solution was applied to the column and the effluent collected in 5-ml reaction vials, frozen and freeze-dried. TMS derivatives were prepared with the addition of 50 μl BSTFA and 100 μl of acetonitrile. The reaction vials were tightly capped and kept at room temperature for 48 h. The vials were then stored at 4 °C. The samples were injected into the GCMS within five days after derivatization.

One g of each digesta sample was hydrolyzed in 100 ml 6 N HCl for 24 h (Blackburn, 1968). Following hydrolyses an aliquot of approximately 250 μl of the hydrolysate was taken, which contained approximately .20 μmol leucine. This aliquot was applied to the anion- and

---

[2]Bio-Rad Laboratories, 32nd & Griffin Ave, Richmond, C.A. 94804

cation-exchange columns in the same manner as described for the blood plasma samples. Further preparation of the digesta samples for GCMS was also similar to that of the blood plasma samples.

The ^{15}N-enrichment analyses were performed on a Varian Vista 6000 gas chromatograph[3] connected to a 7070E Vg analytical organic mass spectrometer[4]. Approximately 1 μl samples were injected into a DB5 gas chromatographic column (.32 mm i.d. x 30 m)[5]. Helium was used as the carrier gas. The flow rate was approximately one ml per sec. The column temperature was kept constant at 140 °C. Inject-port temperature and interface temperatures were 290 and 300 °C, respectively. Electron impact ionization was used for mass spectrometry. The source temperature was 250 °C. The electron impact energy was set at 40 eV. Single ion monitoring was used for ion fragments at mass-charge ratios (m/z) 158.2 and 159.2 corresponding to ^{14}N and ^{15}N ion fragments of both leucine and isoleucine, respectively. Since the molecular weights of the derivatives of leucine and isoleucine are similar, no additional adjustments were neccesary for simultaneous measurement of the enrichment in leucine and isoleucine. The run time was four minutes with a dwell time of 60 msec. Samples were injected every five minutes.

The observed peak areas of the labelled species (^{15}N-leucine and ^{15}N-isoleucine) were corrected for natural abundance according to procedures described by Campbell (1974). Since isotopic enrichment does not have a significant effect on the ionization efficiency, the ^{15}N-enrichment excess in leucine and isoleucine were directly derived from the peak area ratios (Campbell, 1974; Matthews et al., 1981). For

---

[3]Varian, 220 Humboldt Court, Sunnyvale, C.A. 94089
[4]VG Analytical Ltd., Flods Road, Wythenshaw, Manchester, M23 9LE, England
[5]J. & W. Scientific, 91 Blue Ravin Road, Folsom, C.A., 95630

leucine, the ^{15}N-enrichment excess was calculated using the formula:

$$\frac{\text{P.A. m/z 159}}{(1+.003659)\text{xP.A. m/z 158} + \text{P.A. m/z 159}} - \frac{^{15}\text{N-leu exc.}}{^{14}\text{N-leu} + ^{15}\text{N-leu}}$$

P.A. m/z 159 : Peak area at m/z 159, corrected for natural abundance (.14751 x P.A. m/z 158)

P.A. m/z 158 : Peak area at m/z 158

^{15}N-leu exc. : ^{15}N-leucine in sample, corrected for natural abundance in N (.003659 x ^{14}N)

^{14}N-leu : ^{14}N-leucine in sample

^{15}N-leu : ^{15}N-leucine in sample.

The same formula was used to calculate the ^{15}N-enrichment excess in isoleucine. The natural abundance of ^{15}N was determined to be .3673%. It was, therefore, calculated that .3659% (.3673/(1+.3673)) of the peak area at m/z 158 attributed to the peak area at m/z 159 due to natural abundance of ^{15}N. The contributions of natural abundance of isotopes of all atoms present in leucine- and isoleucine-derivatives to the peak area at m/z 159 were determined daily from peak area ratios in unlabelled standards. The determined peak area ratios in the unlabelled standards were very close to those calculated based on the natural abundance of isotopes of the atoms present in derivatized leucine and isoleucine (.14751 of peak area at m/z 158, Campbell, 1974). The formula, however, was adjusted daily for the peak area ratios in the standards.

The contributions of endogenous leucine and isoleucine to total leucine and isoleucine in ileal digesta were calculated in a manner similar to the ^{15}N-isotope dilution technique for measuring

endogenous N (Souffrant et al., 1981, 1986; de Lange et al., 1988c). The ^{15}N-enrichment excess in endogenous leucine (or isoleucine) in digesta was assumed to be similar to that in free plasma leucine (or isoleucine) in the blood samples taken during collection of ileal digesta. The ratio in ^{15}N-enrichment excess in leucine (or isoleucine) in digesta and in the free plasma amino acids indicates the contribution of endogenous leucine (or isoleucine) to total leucine (or isoleucine) in ileal digesta. The endogenous recovery in ileal digesta of the other amino acid(s) and total protein could also be calculated assuming that the amino acid composition of endogenous protein is constant (de Lange et al., 1988a,b). In these calculations, the leucine and isoleucine contents of endogenous protein were assumed to be similar to that observed in pigs fed a protein-free diet and intravenously administered with amino acids simultaneously (de Lange et al., 1988b). Real ileal protein, leucine and isoleucine digestibilities were then calculated from the apparent protein and amino acid digestibilities and the recovery of endogenous protein and amino acids in ileal digesta. The real protein and amino acid digestibilities in these diets were determined simultaneously using the ^{15}N-isotope dilution technique (de Lange et al., 1988c).

Statistical Procedure. The observed ^{15}N-enrichment excesses in each of the different N-containing fractions were analyzed using three way analyses of variance with diet, collection day and animal as sources of variation. The recovery of endogenous protein and amino acids and the calculated real ileal protein and amino acid digestibilities, as determined with each of the isotope dilution techniques, were analyzed

using two way analyses of variance with diet and collection day as sources of variation (Harvey, 1960). Equality of variances for the ^{15}N-enrichment excess in the different N-containing fractions was tested with an F-test using the mean square errors of the ANOVA tables (Steel and Torrie, 1980). The recoveries of endogenous protein and amino acids and the calculated real protein and amino acid digestibilities as determined with the different isotope dilution techniques were analyzed in a similar manner. Treatment means were compared using a t-test for means of samples with unequal variance (Steel and Torrie, 1980).

C. RESULTS AND DISCUSSION

The ^{15}N-enrichment excess in leucine, isoleucine and total N in the TCA-soluble fraction of the blood and in digesta are presented in Table V.1. The ^{15}N-enrichment excess in free plasma leucine was approximately 25 to 30 times higher than in total N of the TCA-soluble fraction of the blood. The ^{15}N-enrichment excess in free plasma isoleucine was about 15 to 25 percent of that in leucine. Matthews et al. (1979), in studies with dogs, showed that the ^{15}N incorporation into plasma amino acids varied largely among the individual amino acids when ^{15}N-leucine was infused continuously. The ^{15}N-enrichment excess in leucine was much higher than in the other amino acids after a nine h infusion period. In addition, alanine, valine, isoleucine, serine, glutamate and ornithine showed a significant incorporation of ^{15}N. ^{15}N-glycine was administered orally to male human adults for a 60 h period in an other study by Matthews et al. (1981). The observed ^{15}N-enrichment excess was highest in glycine, followed by

serine and urea. The studies by Matthews et al. (1979, 1981) illustrate that the ^{15}N-enrichment excess is highest in the amino acid that is used to administer the ^{15}N-isotope, and that transamination among amino acids results in differences in ^{15}N-enrichment excess in urea and plasma amino acids. Some amino acids, like lysine, do not participate significantly in transamination and will show no incorporation of ^{15}N (Matthews et al., 1979, 1981). Because the amino acid composition of endogenous protein is different from the amino acid profile in blood plasma (Wuensche et al., 1987; de Lange et al., 1988a,b; Davey et al., 1973; Keith et al., 1977), the ^{15}N-enrichment excess in total N in the TCA-soluble fraction of the blood may provide an incorrect estimate of the enrichment excess in endogenous protein recovered in digesta collected from the distal ileum. Moreover, ammonia and urea present in blood plasma and in ileal digesta contribute much more to total N in the TCA-soluble fraction of the blood than to endogenous N recovered at the distal ileum. The maximum contribution of N in urea and ammonia to endogenous N in ileal digesta is approximately 25 % (Wuensche et al., 1987; de Lange et al., 1988a,b). The quantity of ammonia in blood plasma contributed approximately 25 % to total N present in the amino acids and ammonia measured in blood plasma of young pigs (Chavez and Bayley, 1977). The urea-N present in plasma of 40 kg growing pigs contributed approximately 40 % to total N present in the amino acids and urea in studies performed by Davey et al. (1973). The ^{15}N-enrichment excess in urea and ammonia in the present study was probably somewhat higher than in the other N-containing compounds in the TCA-soluble fraction of the blood. The ^{15}N-enrichment excess was higher in urine than in the

TCA-soluble fraction of the blood (Figure IV.1). Urea and ammonia N accounted for approximately 85 to 95 % of N excreted in urine (Reeds et al., 1980).

The ^{15}N-enrichment excess in leucine and isoleucine was more variable than that in total N in the TCA-soluble fraction of the blood (Table V.1); probably as a result of the shorter turnover times of the free leucine and isoleucine than of the N pool. Fuerst (1983) indicated that the turnover time of the free leucine pool is about ten times shorter than of all the free amino acids combined in adult human subjects. The turnover time of the free leucine and isoleucine pools were estimated to be .8 and .7 h, respectively. On the other hand, the urea pool, the single largest contributor to N in the TCA-soluble fraction of blood, shows a relatively long turnover time. Mosenthin (1987) estimated that the turnover time of the urea pool in pigs with a bodyweight of approximately 80 kg was between 7.75 and 8.50 h. Short time changes in the turnover rate of the pools of free leucine, free isoleucine or total N in the TCA-soluble fraction of the blood will, therefore, have a larger effect on the ^{15}N-enrichment excess in leucine, and probably isoleucine, than in total N. In order to reduce the variation in the estimated ^{15}N-enrichment excess in free plasma leucine and isoleucine, blood should be sampled more frequently.

The recoveries of endogenous leucine, isoleucine and protein in digesta collected from the distal ileum in pigs, as determined with their respective isotope dilution techniques, are presented in table V.2. The recoveries of the other amino acid(s) or protein, within the columns were indirectly calculated assuming a constant amino acid composition of endogenous protein. The values for protein obtained

with the ^{15}N-leucine isotope dilution technique (7.1 to 11.0 g per kg dry matter intake) were considerably lower ($P<.05$) than those with the ^{15}N-isotope (25.5 to 30.5 g per kg dry matter intake) and the ^{15}N-isoleucine isotope dilution techniques (21.8 to 24.9 g per kg dry matter intake). The values obtained with the ^{15}N-leucine isotope dilution technique were even lower than those in studies in which pigs were simply fed protein-free diets (Wuensche et al., 1987; de Lange et al., 1988a,b). As a result, the observed real ileal amino acid and protein digestibilities in the diets differed largely for the different isotope dilution techniques (Table V.3). The highest real ileal protein and amino acid digestibilities were obtained with the ^{15}N-isotope dilution techniques followed by the ^{15}N-isoleucine and the ^{15}N-leucine isotope dilution techniques, respectively. Several factors may be responsible for the differences between the recoveries in protein, leucine and isoleucine in ileal digesta.

A possible diurnal variation in ^{15}N-enrichment excess in free plasma leucine (or isoleucine) could not be determined because of the pattern of blood sampling that was used in the present study. Since the blood samples were taken during feeding, the animals were always in the same physiological state during sampling. The appearance of dietary amino acids in the portal blood is at a minimum during feeding (Rerat, 1985). The amino acids in the TCA-soluble fraction of blood are diluted to a lesser extent by recently absorbed dietary amino acids during feeding as compared to three to five h after feeding when large quantities of dietary amino acids enter the portal blood (Rerat, 1985). Therefore, the ^{15}N-enrichment excess in leucine and isoleucine in the blood samples taken during feeding most likely

provide an overestimation of the average ^{15}N-enrichment excess in leucine and isoleucine in the TCA-soluble fraction of blood. As a result, the contributions of endogenous leucine and isoleucine to total leucine and isoleucine in digesta were most likely underestimated.

The question whether the ^{15}N-enrichment excess in leucine (or isoleucine) in the TCA-soluble fraction of blood is a valid indicator of that of leucine (or isoleucine) in endogenous protein that is secreted into the digestive tract also deserves consideration. This question is especially critical for endogenous protein that originates from mucosal cells. In adult rats and rabbits, amino acid transport through mucosal cells to enterocytes is shown to be restricted to the villus tips while the mucosal cells are produced in the crypt compartment (Menge et al., 1982; Smith and Syme, 1982; Syme and Smith, 1982). Protein synthesis within the mucosal cells takes place as the cells migrate from the crypts to the tops of the villi. The mucosal cells are shedded from the tips of the villi into the lumen of the digestive tract. Alpers (1972) clearly showed that amino acids from the gastro-intestinal lumen are directly incorporated into protein in intestinal tissue without having to enter the main blood circulation. The relative contribution of leucine and isoleucine that is directly absorbed from the gastro-intestinal lumen and of leucine and isoleucine derived from the main blood circulation to endogenous protein secreted into the gastro-intestinal tract remains to be elucidated. Because of this dilution, the actual contribution of endogenous leucine and isoleucine to leucine and isoleucine in the digestive tract, based on the ^{15}N-enrichment excess in leucine and isoleucine in the TCA-soluble fraction of blood and in digesta, would be underestimated.

The aforementioned factors both lead to an underestimation of the recovery of endogenous protein and amino acids in digesta collected from the distal ileum in pigs. Theoretically, these underestimations should be approximately similar for the ^{15}N-leucine and the ^{15}N-isoleucine isotope dilution technique. The difference in apparent ileal digestibilities between leucine and isoleucine in the feedstuffs tested in the present study varied between .1 and 2.9 percentage units (de Lange et al., 1988c). The leucine to isoleucine ratio in endogenous protein is relatively constant (de Lange et al., 1988a,b) and similar to that in the feedstuffs tested (de Lange et al., 1988c). Moreover, leucine and isoleucine share the same transport system for absorption from the intestinal lumen which does not allow for an independant control of leucine and isoleucine absorption (Karasov et al., 1987). It appears that the observed recovery of endogenous protein and amino acids is a function of the level of ^{15}N-enrichment excess in the precursor amino acid pool. Transamination between amino acids in the intestinal tissue, which would not be reflected in the ^{15}N-enrichment excess in the free plasma amino acids, could lead to a smaller difference between the ^{15}N-enrichment excess in leucine and isoleucine that is used for protein synthesis in intestinal tissue than in the free plasma amino acids. Intestinal tissue, however, is not an active site for transamination among branched chain amino acids (Harper et al., 1985).

The microflora in the stomach and small intestine may also have contributed to transamination between amino acids. Several studies indicated that, 25 to 30 % of the total amount of N in digesta recovered from the distal ileum may be of bacterial origin (Dierick et

al., 1983; Poppe et al., 1983; Drochner, 1984). Studies with ruminants showed that, endogenous N and especially urea-N, which is secreted into the gastro-intestinal lumen, may be incorporated into microbial protein (Kennedy and Milligan, 1980). In pigs substantial amounts of urea are secreted into the small intestine (Mosenthin, 1987). The relative contribution of endogenous amino acids, urea (and possibly ammonia) and of dietary N to microbial N in ileal digesta remains to be elucidated. The ^{15}N-enrichment excess in endogenous N that is incorporated into leucine during microbial protein synthesis will be lower than in endogenous leucine directly derived from blood plasma. If the contribution of microbial endogenous N to total leucine in ileal digesta is significant, the contribution of endogenous leucine to total leucine will be underestimated when the ^{15}N-leucine dilution technique is used. Because the ^{15}N-enrichment excess in isoleucine in the TCA-soluble fraction of the blood was lower than in leucine and closer to that in N in the TCA-soluble fraction of the blood, this underestimation would be smaller for isoleucine than for leucine (Table V.2).

As can be inferred from the previous discussion, disputable results may be obtained with an ^{15}N-isotope dilution technique, in which the individual amino acids are used, for determining the recovery of endogenous protein and amino acids in ileal digesta. The results of the present study illustrate the complexity when such a method is used. The ^{14}C-amino acid isotope dilution technique will possibly overcome some of these problems. This technique, however, will not account for the contribution of endogenous N incorporated into microbial protein and will, therefore, also lead to an underestimation

of the recovery of endogenous N in digesta collected from the distal ileum. The best method would be the one in which all the endogenous N sources will be uniformally labelled. In order to achieve this, a complete mixture of ^{15}N-labelled amino acids should be administered intravenously. However, such a method would be very expensive and, therefore, impractical, even for experimental purposes. The alternative to the aforementioned method, is an ^{15}N-isotope dilution technique in which an amino acid, actively involved in transamination (such as leucine), is infused continuously followed by measurement of the ^{15}N-enrichment excess in total N in the precursor pool for endogenous protein synthesis and in digesta collected from the distal ileum. Since endogenous protein that is secreted into the intestinal lumen is synthesized in several distinct organs, the N in the de-proteinized fraction of the blood is probably the best defined precursor pool for endogenous protein synthesis. However, the presence of microbial fermentation in the small intestine could theoretically lead to an overestimation of the recovery of endogenous protein in ileal digesta when this ^{15}N-isotope dilution technique is used. If the microbes preferentially incorporate endogenous N from urea (and possibly ammonia) into microbial protein, the ^{15}N-enrichment excess in this microbial fraction of endogenous protein could be higher than in the TCA-soluble fraction of the blood. In that situation, plasma urea (and possibly ammonia) should be considered the precurser pool for bacterial protein synthesis and possibly for endogenous protein synthesis. All other factors previously referred to would probably lead to an underestimation of the recovery of endogenous protein at the distal ileum.

In conclusion, the results of the present study appear to support the ^{15}N-isotope dilution technique, as proposed by Souffrant et al. (1981, 1986), for determining the (minimum) recovery of endogenous protein in digesta collected from the distal ileum in pigs. If assumptions are made concerning the amino acid composition of endogenous protein, the ^{15}N-isotope dilution technique can also be used for determining the (minimum) recovery of endogenous amino acids in digesta collected from the distal ileum in pigs.

D. BIBLIOGRAPHY

Alpers, D. H. 1972. Protein synthesis in intestinal mucosa: the effect of route administration of precursor amino acids. J. Clin. Invest. 51: 167.

Blackburn, S. 1968. Amino acid determination methods and techniques. Marcel Dekker Inc., New York.

Campbell, I. M. 1974. Review: Incorporation and dilution values: Their calculation in mass spectrally assayed stable isotope labelling experiments. Bioorg. Chem. 3: 386.

Chavez, E. R. and H. S. Bayley. 1977. Variations in the concentration of blood plasma free amino acids in suckled and fasted neonatal pigs. J. Anim. Sci. 44: 242.

Davey, R. J., J. W. Phelps and C. H. Thomas. 1973. Plasma free amino acids of swine as influenced by diet protein level, animal age and time of sampling. J. Anim. Sci. 37: 81.

de Lange, C. F. M., W. C. Sauer, R. Mosenthin and W. B. Souffrant. 1988a. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. (In press), (This thesis, Chapter II).

de Lange, C. F. M., W. C. Sauer and W. B. Souffrant. 1988b. The effect of the protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. J. Anim. Sci. (In press), (This thesis, Chapter III).

de Lange, C. F. M., W. B. Souffrant and W. C. Sauer. 1988c. Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N-isotope dilution technique. J.

Anim. Sci. (In preparation), (This thesis, Chapter IV).

Dierick, N. A., J. A. Decuypere, J. Lannoye, I. Vervaeke and H. K. Henderickx. 1983. Digestion ileale et fecale de la matiere seche, des proteines et des acides amines de quelques rations conventionelles pour les porcs a l'engrais. Rev. Agric. (Bruxelles), 36: 1713.

Drochner, W. 1984. The influence of changing amounts of crude fibre and pectic components on prececal and postileal digestive processes in the growing pig. J. Anim. Physiol. Anim. Nutr. 14: 1.

Fuerst, P. 1983. Intracellular muscle free amino acids-their measurement and function. Proc. Nutr. Soc. 42: 451.

Harper, A. E., R. H. Miller and K. P. Block. 1984. Branched chain amino acid metabolism. Ann. Rev. Nutr. 4: 409.

Harvey, W. R. 1960. Least square analyses of data with unequal subclass numbers. USDA publ. ARS 20-8.

Haymond, M. W., C. P. Howard, J. M. Miles and J. E. Gerich. 1980. Determination of leucine flux in vivo by gas chromatography- mass spectrometry utilizing stable isotopes for trace and internal standard. J. Chromatogr. 183: 403.

Karasov, W. H., D. H. Solberg and J. M. Diamond. 1987. Dependance of intestinal amino acid uptake on dietary protein or amino acid levels. Am. J. Physiol. 252: G614.

Keith, M. O., H. G. Botting and R. W. Peace. 1977. Dietary effects on the concentrations of free amino acids in plasma and whole blood of pigs. Can. J. Anim. Sci. 57: 295.

Kennedy, P. M. and L. P. Milligan. 1980. The degradation and utilization of endogenous urea in the gastro-intestinal tract of

ruminants. A Review. Can. J. Anim. Sci. 60: 205.

Krishnamurti, C. R. and A. L. Schaefer. 1987. Measurement of plasma flux and protein synthesis in pregnant ewes using gas chromatography and mass spectrometry. Nutr. Rep. Intern. 35: 683.

Low, A. G. 1982. Digestibility and availability of amino acids from feedstuffs for pigs: a review. Livestock Prod. Sci. 9: 511.

Matthews, D. E., E. B. Ben-Galim and D. M. Bier. 1979. Determination of stable isotopic enrichment in individual plasma amino acids by chemical ionization-mass spectrometry. Anal. Chem. 51: 80.

Matthews, D. E., J. M. Conway, V. R. Young and D. M. Bier. 1981. Glycine nitrogen metabolism in man. Metabolism 30: 886.

Menge, H., R. Hopert, Alexopoulos and E. O. Riecken. 1982. Three-dimensial structure and cell kinetics at different sites of rat intestinal remnants during the early adaptive response to resection. Res. Exp. Med. 181: 77.

Mosenthin, R. 1987. Untersuchungen zum Einfluss pflanzlicher Kohlenhydrate in Rationen wachsender Schweine auf die endogene Stickstoff- und Enzymsekretion in den Verdauungstrakt sowie auf die praecaecale und postileale Umsetzungen N-haltiger Verbindungen. Habilitationsschrift. Universitaet Kiel, F. R. G.

Poppe, S., H. Meier, H. J. Benneke and E. Stuewe. 1983. Zur Protein- und Aminosaeuren Verdaulichkeit in verschiedenen Darmabschnitten beim Schwein. Arch. Tierernaehr. 33: 743.

Reeds, P. J., A. Cadenhead, M. F. Fuller, G. E. Lobley and J. D. McDonald. 1980. Protein turnover in growing pigs. Effects of age and food intake. Brit. J. Nutr. 43: 445.

Rerat, A. A. 1985. Intestinal absorption of end products of digestion

of carbohydrates and proteins in pigs. Arch. Tierernaehr. 35: 461.

Sauer, W. C. and L. Ozimek. 1986. Digestibility of amino acids in swine: results and their practical application. A Review. Livestock Prod. Sci. 15: 367.

Smith, M. W. and G. Syme. 1982. Functional differentiation of enterocytes in the follicle-associated epithelium of rat Peyer's patch. J. Cell Sci. 55: 147.

Souffrant, W. B., R. Koehler, R. Matkowitz, G. Gebhardt and H. Schmandke. 1981. Ernaehrungsphysiologische Untersuchungen an Schweinen zur Beurteilung von modifizierten Proteinen. Arch. Tierernaehr. 31: 672.

Souffrant, W. B., B. Darcy-Vrillon, T. Corring, J. P. Laplace, R. Koehler, G. Gebhardt and A. Rerat. 1986. Recycling of endogenous nitrogen in the pig. Arch. Tierernaehr. 36: 269.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of statistics. McGraw-Hill Book Co., New York.

Syme, G. and M. W. Smith. 1982. Intestinal adaptation to protein deficiency. Biol. Int. Rep. 6: 573.

Wuensche, J., U. Hermann, M. Meinl, U. Hennig, F. Kreienbring and P. Zwierz. 1987. Einfluss exogener Faktoren auf die praezaekale Naehrstoff und Aminosaeurenresorption ermittelt an Schweinen mit Ileo-Rektal-Anastomosen. Arch. Tierernaehr. 32: 431.

Table V.1. The ^{15}N-enrichment excess (%) in total N, leucine and isoleucine in the TCA-soluble fraction of blood and in digesta collected from the distal ileum in pigs fed the experimental diets and continuously administered ^{15}N-leucine intravenously.

	^{15}N-enrichment (%) excess in:		
	total N	Leucine	Isoleucine
	TCA-soluble fraction of blood[a]:		
Soybean meal	.16[f]	4.15[d]	.98[e]
Canola meal	.16[f]	4.04[d]	1.04[e]
Wheat	.18[f]	5.19[d]	.88[e]
Barley	.21[f]	5.96[d]	.89[e]
SE[b]	.003[f]	.467[d]	.065[e]
Coef. of var.[c]	1.7	10.4	6.8
	ileal digesta:		
Soybean meal	.13[f]	.88[d]	.59[e]
Canola meal	.08[f]	.62[d]	.40[e]
wheat	.18[f]	1.25[d]	.70[e]
Barley	.17[f]	1.06[d]	.56[e]
SE[b]	.003[e]	.057[d]	.048[d]
Coef. of var.[c]	2.1	10.4	8.5

[a]Based on values in blood samples taken during the collection of digesta from the distal ileum on d seven and nine of the infusion period.
[b]Standard error of the four means within each column.
[c]Coefficient of variation of the four means in one column.
[d,e,f]Values in the same row followed by different superscripts differ significantly ($P<.05$).

Table V.2. The recovery of endogenous protein, leucine and isoleucine (g per kg dry matter intake) in digesta collected from the distal ileum in pigs, fed the experimental diets and continuously administered ^{15}N-leucine, and as determined with different isotope dilution techniques.

	Soybean meal	Canola meal	Wheat	Barley	SE[a]
Protein					
^{15}N[b]	25.5[e]	30.5[e]	27.4[e]	27.7[e]	2.37[f]
^{15}N-leu[c]	9.0[f]	11.0[f]	7.7[f]	7.1[f]	1.58[g]
^{15}N-ileu[d]	23.0[e]	24.9[e]	21.8[e]	22.2[e]	4.34[e]
Leucine					
^{15}N[b]	1.3[e]	1.6[e]	1.4[e]	1.4[e]	.12[f]
^{15}N-leu[c]	.5[f]	.6[f]	.4[f]	.4[f]	.08[g]
^{15}N-ileu[d]	1.2[e]	1.3[e]	1.1[e]	1.2[e]	.22[e]
Isoleucine					
^{15}N[b]	.9[e]	1.0[e]	.9[e]	.9[e]	.08[f]
^{15}N-leu[c]	.3[f]	.4[f]	.3[f]	.2[f]	.05[g]
^{15}N-ileu[d]	.8[e]	.8[e]	.7[e]	.7[e]	.15[e]

[a] Standard error of the means.
[b] ^{15}N-isotope dilution technique.
[c] ^{15}N-leucine isotope dilution technique.
[d] ^{15}N-isoleucine isotope dilution technique.
[e,f,g] Values within a column followed by different superscripts differ significantly ($P<.05$).

Table V.3. Real ileal protein, leucine and isoleucine digestibilities (%) in the experimental diets determined with different isotope dilution techniques.

	Soybean meal	Canola meal	Wheat	Barley	SE[a]
Protein					
^{15}N[b]	97.5[e]	84.1[e]	99.0[e]	94.2[e]	1.05[g]
^{15}N-leu[c]	88.7[f]	72.5[f]	85.3[f]	75.8[f]	1.69[f]
^{15}N-ileu[d]	96.2[e]	80.7[e]	95.2[e]	89.3[e]	3.18[e]
Leucine					
^{15}N[b]	94.5[e]	84.1[e]	98.8[e]	94.3[e]	1.07[f]
^{15}N-leu[c]	88.7[f]	76.7[f]	88.9[f]	81.8[f]	1.06[f]
^{15}N-ileu[d]	93.7[e]	82.3[e]	96.0[e]	91.0[e]	2.17[e]
Isoleucine					
^{15}N[b]	95.4[e]	82.9[e]	99.5[e]	94.9[e]	1.22[f]
^{15}N-leu[c]	89.2[f]	74.2[f]	87.9[f]	80.1[f]	1.17[f]
^{15}N-ileu[d]	94.5[e]	80.4[e]	96.2[e]	91.0[e]	2.45[e]

[a]Standard error of the means.
[b] ^{15}N-isotope dilution technique.
[c] ^{15}N-leucine isotope dilution technique.
[d] ^{15}N-isoleucine isotope dilution technique.
[e,f,g]Values in a column of three values followed by different superscripts differ significantly ($P<.05$).

VI. GENERAL DISCUSSION AND CONCLUSIONS

The most commonly used method for determining the recovery of endogenous protein and amino acids in digesta collected from the distal ileum in pigs is by feeding protein-free diets (direct method; Wuensche et al., 1987). A protein-free diet is usually fed in combination with protein-containing diets to determine the true protein and amino acid digestibilities in the protein containing diets. It is assumed that the recovery of endogenous protein and amino acids in digesta is similar between pigs fed the protein-free and the protein-containing diet. When different diets with varying protein content are fed for determining the recovery of endogenous protein and amino acids in ileal digesta (regression method) it is also assumed that there is no relationship between protein intake and the recovery of endogenous protein and amino acids in ileal digesta (Carlson and Bayley, 1970). The validity of these assumptions should be questioned. Krawielitzki et al. (1977), in studies with rats, showed a positive linear relationship between the level of inclusion of protein-containing feedstuffs and the excretion of endogenous protein in feces. Three distinct questions can be raised in evaluating the direct and regression method for determining the recovery of endogenous protein and amino acids in ileal digesta. First of all, it can be questioned whether the recovery of endogenous protein and amino acids is affected by components other than protein in the diet. Secondly, the protein status of the animal may affect the recovery of endogenous protein and amino acids in ileal digesta. And thirdly, the presence of dietary protein in the digestive tract as such may affect the endogenous

protein and amino acid recoveries. Three experiments were carried out to investigate these questions.

The results of the first experiment indicate that the composition of the protein-free diet affects the recovery of endogenous protein and amino acids in ileal digesta and feces in pigs. In particular, the inclusion of four percent pectin in the protein-free diet increased the recovery of endogenous protein in ileal digesta: from 19.8 to 24.0 g per kg dry matter intake. Sauer et al. (1977) and Taverner et al. (1981) also observed that the recovery of endogenous protein in ileal digesta was affected by the composition of the protein-free diet. The differences in the recoveries of endogenous protein in ileal digesta were largely due to differences in the recoveries of arginine, glycine and, especially, proline. Only small differences were observed in the true digestibilities of amino acids that may be considered critical in diet formulation (lysine, methionine and threonine) when the recoveries of protein and amino acids in ileal digesta were used to calculate true digestibilities in a barley and a barley-soybean meal diet. Apparently, the composition of the protein-free diet itself is not a critical factor for determining the recovery of these amino acids in ileal digesta in pigs.

In the second experiment, the effect of the protein status of the pig on the recovery of endogenous protein in digesta collected from the distal ileum was studied. The intravenous administration of a well-balanced mixture of amino acids reduced the recovery of endogenous protein in ileal digesta from 18.5 to 12.7 g per kg dry matter intake. Of the amino acids, only the recovery of endogenous proline was reduced ($P<.05$). When the recoveries of protein and amino acids in ileal

digesta were used to calculate the true digestibilities in a barley and a barley-soybean meal diet only small differences were observed for lysine, methionine and threonine. The true proline digestibilities in these diets were affected to a large extent. When the recoveries of endogenous amino acids, observed in pigs fed the protein-free diet only, were used in these calculations the true proline recovery exceeded 100%, which shows that when the protein-free diet was fed the recovery of proline in ileal digesta was higher than when protein-containing diets were fed. Sauer et al. (1977) also stated that feeding protein-free diets may provide a higher estimate of the recovery of proline, and in some cases of glycine, in ileal digesta as compared to when protein containing diets are fed. The intravenous administration of amino acids reduced the recovery of proline, in particular, in ileal digesta. On the other hand, the recovery of endogenous protein is probably underestimated when a protein-free diet is fed (Krawielitzki et al., 1977). The amino acid composition of endogenous protein in ileal digesta determined in pigs fed a protein-free diet and parentally administered with amino acids will probably provide a better estimate of the amino acid composition of endogenous protein in digesta of pigs fed protein-containing diets than the composition of endogenous protein in ileal digesta in pigs fed a protein-free diet only.

In the third study, the ^{15}N-isotope dilution technique was used for determining the recovery of endogenous protein in digesta collected from the distal ileum in pigs fed protein-containing diets (Souffrant et al., 1981, 1986). Endogenous protein, secreted into the gastro-intestinal tract, was labelled via a continuous intravenous

infusion of ^{15}N-leucine. The labelling of endogenous protein allows for the differentiation between non-digested dietary and endogenous protein in the digestive tract. The recoveries of endogenous protein at the distal ileum were 25.5, 30.5, 27.4 and 27.7 g per kg dry matter intake for pigs fed diets in which soybean meal, canola meal, wheat or barley were included as the sole protein source, respectively. These values were higher than those observed in pigs fed protein-free diets (exp. 1) and those summarized by Wuensche et al. (1987) and in pigs fed a protein-free diet and simultaneously administered amino acids intravenously (exp. 2). The real amino acid digestibilities were calculated from the apparent and real ileal protein digestibilities and the apparent ileal amino acid digestibilities. The amino acid composition of endogenous protein was assumed to be similar to that observed in pigs fed a protein-free diet and parentally administered with amino acids simultaneously. The real ileal protein and amino acid digestibilities were higher than the indirectly calculated true digestibilities in these feedstuffs, based on feeding protein-free diets to determine the recoveries of endogenous protein and amino acids at the distal ileum.

The results of the ^{15}N-isotope dilution technique were compared to those obtained with alternative and newly introduced ^{15}N-leucine and ^{15}N-isoleucine isotope dilution techniques. There appeared to be a negative relationship between the level of ^{15}N-enrichment excess in leucine, isoleucine and total N in the TCA-soluble fraction of blood on the one hand, and the recovery of endogenous amino acids and protein, as determined with their respective isotope dilution techniques on the other hand. The ^{15}N-enrichment excess in free plasma leucine and

isoleucine was probably higher than in the endogenous leucine and isoleucine recovered in ileal digesta. This was probably due to transamination among amino acids, either in the intestinal tissue or as a result of microbial fermentation in the intestinal lumen, and to dilution of endogenous amino acids with dietary amino acids directly incorporated into endogenous protein in intestinal tissue. Of the three isotope dilution techniques that were compared, the ^{15}N-isotope dilution technique probably provided the most reliable results.

The results of the ^{15}N-isotope dilution technique showed that differences in apparent ileal protein and amino acid digestibilities between the feedstuffs were largely due to differences in the recovery of endogenous protein and amino acids in ileal digesta and not to differences in real ileal protein and amino acid digestibilities. It is, therefore, important to identify the dietary factors that are responsible for the differences in the recovery of endogenous protein and amino acids when different protein-containing diets are fed, such as protein intake, protein quality and structure, level and source of fiber and anti-nutritional factors (Sauer and Ozimek, 1986). Manipulation or removal of these factors from the diets should result in an improvement in protein and amino acid utilization.

Possible interactions between apparent ileal protein and amino acid digestibilities among different feedstuffs, as was discussed by Imbeah et al. (1988), could probably be attributed more to endogenous protein and amino acid recoveries rather than to real ileal protein and amino acid digestibilities. Interactions between ileal protein and amino acid digestibilities among feedstuffs implicates that the apparent ileal protein and amino acid digestibilities in a mixture of

feedstuffs can not be predicted directly from those in the individual feedstuffs. Endogenous protein contributes a large proportion to total protein recovered in ileal digesta and this quantity is possibly affected by various dietary components. This may become an important consideration in diet formulation. However, the results of the present study indicate that there were no differences in the recovery of endogenous protein, expressed as g per kg dry matter intake, when the different protein-containing diets were fed. It is therefore unlikely that there are interactions between apparent ileal protein digestibilities among the feedstuffs tested in these studies.

The present studies indicate that the direct method and the regression method are invalid for the determination of the recovery of endogenous protein and amino acids in digesta collected from the distal ileum in pigs. The ^{15}N-isotope dilution technique showed that the recovery of endogenous protein in ileal digesta was larger than previously determined with the direct and regression methods. Therefore, studies on factors that affect the recovery of endogenous protein in ileal digesta in pigs, using the ^{15}N-isotope dilution technique, deserve more attention in future research.

A. BIBLIOGRAPHY

Carlson, K. H. and H. S. Bayley. 1970. Nitrogen and amino acids in the feces of young pigs receiving a protein-free diet and diets containing graded levels of soybean meal or casein. J. Nutr. 100: 1353.

Imbeah, M., W. C. Sauer and R. Mosenthin. 1988. The prediction of the digestible amino acid supply in barley-soybean meal or canola meal diets and pancreatic secretions in pigs. J. Anim. Sci. 66: 1409.

Krawielitzki, K., T. Volker, S. Smulikowska, H. D. Bock and J. Wuensche. 1977. Weitere Untersuchungen zum Multikompartment-Modell des Protein-stoffwechsels. Arch. Tierernaehr. 27: 609.

Sauer, W. C., S. C. Stothers and R. J. Parker. 1977. Apparent and true availabilities of amino acids in wheat and milling by-products for growing pigs. Can. J. Anim. Sci. 57: 775.

Sauer, W. C. and L. Ozimek. 1986. Digestibility of amino acids in swine: results and their practical application. A review.

Souffrant, W. B., R. Koehler, R. Matkowitz, G. Gebhardt and H. Schmandke. 1981. Ernaehrungsphysiologische Untersuchungen an Schweinen zur beurteilung von modifizierten Proteinen. Arch. Tierernaehr. 31. 675.

Souffrant, W. B., B. Darcy-Vrillon, T. Corring, J. P. Laplace, R. Koehler, G. Gebhardt and A. Rerat. 1986. Recycling of endogenous nitrogen in the pig. Arch. Tierernaehr. 36: 269.

Taverner, M. R., I. D. Hume and D. J. Farell. 1981. Availability to pigs of amino acids in cereal grains 1: Endogenous levels of amino acids in ileal digesta and faeces of pigs given cereal diets.

Brit. J. Nutr. 46: 149.

Wuensche, J., U. Hermann, M. Meinl, U. Hennig, F. Kreienbring and P. Zwierz. 1987. Einfluss exogener Faktoren auf die praezaekale Naehrstoff und Aminosaeurenresorption ermittelt an Schweinen mit Ileo-Rektal-Anastomosen. Arch. Tierernaehr. 32: 431.