ILEAL RECOVERY OF ENDOGENOUS AMINO ACIDS IN PIGS

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ILEAL RECOVERY OF ENDOGENOUS AMINO ACIDS IN PIGS

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Stellingen

1. The homoarginine ratio method is a good approach to determine qualitative estimates of endogenous amino acids recovered from the terminal ileum of pigs. *This thesis.*

2. The increase in apparent ileal amino acid digestibilities of soybean meal in piglets after weaning is due to a decrease in the ileal recoveries of endogenous amino acids rather than an increase in the true ileal amino acid digestibilities of the soybean meal. *This thesis.*

3. The bacterial flora in the small intestine of newly weaned piglets increases with time after weaning and changes the amino acid composition of protein recovered from the terminal ileum. *This thesis.*

4. The bacterial flora in the small intestine of pigs acts as a nitrogen-sink by assimilating dietary and endogenous amino acids, thereby, making them less available for digestion and absorption. *This thesis.*

5. A substantial proportion of dietary amino acids are absorbed and transported as plasma peptides in the portal vein blood of pigs. *This thesis.*

6. In contrast to the present situation, tomorrow's animal nutritionists will take a holistic approach and emphasize diminishing return rather than maximum end product as a research objective.

7. Scientific knowledge is increased by scientists that report qualitative speculation about that which is unknown than by those that report redundant proof of that which is established fact.

8. The time has come for eukaryotes to work with instead of against prokaryotes.

9. Development of sustainable animal production in the Netherlands has encouraged some Dutch farmers to establish their farms in Canada. However, the future of animal production in Canada is also best served by sustainability.

10. There is twice as much bureaucracy in the Netherlands compared to Canada, however, things get done twice as fast.

11. The continuous extinction of many living species from this earth is a direct consequence of the proliferation of a single species; *homo sapiens.*

William R. Caine
Ileal recovery of endogenous amino acids in pigs
Wageningen, 18 December 1997.

dedicated to J.A.C. and Alice and Bob

Things without all remedy should be without regard.
William Shakespeare

Preface

The contents of this book represents more of a "Life Experience" than a "Scientific Endeavour". To family members and the many friends and colleagues, especially those of Veevoeding and TNO-ILOB, whose support and encouragement made this an enjoyable experience:

THANK-YOU.

To the three distinguished Professors that supervised the gathering of information contained in this book,
Willem Sauer for his guidance and faith in me;
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THANK-YOU.

Caine, W.R. 1997. Ileal Recovery of Endogenous Amino Acids In Pigs. Ileal recovery of endogenous amino acids is important for determining balanced homeostasis of protein metabolism in pigs and the true digestibility of dietary protein. In this context, the ileal recoveries of endogenous amino acids were determined in growing pigs fed guanidinated Nutrisoy protein test meals with low and high concentration of soybean trypsin inhibitors (SBTI) using the homoarginine ratio method. Although, there were some differences in the amino acid composition between guanidinated and unguanidinated Nutrisoy the homoarginine ratio method was an effective approach to determine qualitative increases in endogenous recoveries of amino acids when the protein test meals had a higher concentration of SBTI. The *in vitro* incubation of soybean meal with a commercial protease increased protein solubility and decreased the content of SBTI. However, neither apparent or true ileal amino acid digestibilities were improved in newly weaned piglets fed protease-treated soybean meal. Recoveries of endogenous branched-chain and aromatic amino acids were higher on d 6 to 7 than d 15 to 16 after weaning, suggesting dietary change- and/or age-dependent adaptive increases in the secretions of pepsin and pancreatic proteases. In contrast, bacterial contributions to total and endogenous recoveries of nitrogen and amino acids in ileal digesta of the piglets increased with time after weaning. In this respect, the enterobacteriaceae act as a nitrogen-sink by assimilating available dietary and endogenous amino acids, thereby, making them unavailable for absorption by the piglets. Total exchange of amino acids across the portal vein-drained tissue of pigs fed a cornstarch-based wheat gluten diet was 55.8 and 130.2 mmol/h for the plasma free amino acid and plasma peptide pools, respectively. The corresponding numerical estimate of 77.6 mmol/h for red blood cells was statistically not significant ($P > 0.1$) because of large standard errors from accumulated analytical variation. Similar amino acid profiles of wheat gluten and the plasma peptide pool indicate that a substantial proportion of dietary amino acids were exchanged into and transported by the portal vein blood in the form of plasma peptides. The high content of serine and threonine (intestinal mucus) and glutamate, aspartate and the branched-chain amino acids (pancreatic secretions) suggests that some plasma peptides were of endogenous origin. The exchange of amino acid across the portal vein-drained tissue of pigs is a dynamic process that involves the plasma free amino acid and plasma peptide pools and probably red blood cells.

PhD thesis, Department of Animal Nutrition, Wageningen Institute of Animal Sciences, Wageningen Agricultural University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands.

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General Introduction

Perhaps, the most challenging problem in the field of swine nutrition is the development of an effective technique to determine the availability of amino acids in dietary protein sources. An adequate level of dietary amino acids, together with a balanced energy intake, must be provided to the growing pig so that a maximum lean tissue growth is realized (Bikker, 1994). In particular, the requirements of amino acids that may be limiting the growth of the pig must be evaluated. Feeding excessive dietary protein to meet the amino acid requirements of pigs results in an undue environmental burden of waste nitrogen in feces and urine (Huisman et al. 1993).

Endogenous Protein Secretions

Endogenous protein originates from digestive enzymes (salivary, pancreatic and mucosal), gastric and bile secretions, sloughed epithelial cells and mucus secretions throughout the digestive tract. Various studies have been carried out to measure the secretions from these sources. These include saliva (Corring, 1980), gastric secretions (Zebrowska et al. 1983), bile secretions (Juste et al. 1983) and pancreatic secretions (Corring et al. 1972; Hee et al. 1985; Gabert et al. 1996; Li et al. 1997). There is limited information on endogenous secretions from the small intestine and the factors that influence these secretions. Auclair (1986) summarized results from the literature and reported the following range of estimates for endogenous nitrogen secretion (g/d): salivary and gastric secretions, 2.0 to 3.3; pancreatic secretion, 2.5 to 6.7; bile secretion, 1.8 to 3.0; small intestinal secretion, 14.4 and sloughed cells, 1.4 to 2.0. Pancreatic and small intestinal secretions make the largest contributions to total endogenous nitrogen secretion at 17 and 57%, respectively.

i) Pancreatic Protein Secretions

The importance of the pancreatic secretions for digestion has been well documented (e.g. Corring, 1980; Makkink and Verstegen, 1990). The secretion of nitrogen in pancreatic juice ranges from 2.5 to 6.7 g/d (Auclair, 1986). Approximately 60 to 80% of this nitrogen is in the form of protein that has a relatively high content of aspartate, glutamate and the branched-chain amino acids (Corring and Jung, 1972; Gabert et al. 1996). A large proportion of the nonprotein nitrogen in pancreatic juice originates from urea (Mosenthin et al. 1992). Pancreatic secretions have been measured *in vivo* by direct cannulation of the pancreatic duct (Corring et. al. 1972) and the pouch method (Hee et al. 1985). Many studies have been carried out on dietary factors that influence pancreatic secretions. Changes in the dietary content of starch,

fat and protein have an affect on the pancreatic secretion of nitrogen and enzymes in pigs (Hee et al. 1988a). Feeding pigs more frequently increases pancreatic enzyme secretions (Hee et al. 1988b). On the other hand, the inclusion of different levels and sources of fiber influenced pancreatic protein secretion but not enzyme activities (Zebrowska and Low, 1987). Concentration and composition of amino acids in pancreatic juice of pigs was also not affected by diets containing high levels of tannins (Gabert et al. 1996) and soybean trypsin inhibitors (Li et al. 1997).

ii) Small Intestinal Protein Secretions

The small intestinal secretions include mucus, digestive enzymes, sloughed cells and urea. Of these, mucus contributes the largest proportion of nitrogen (Auclair, 1986). Mucus forms the slippery gel covering the mucosal surface of the gastrointestinal tract. Several functions, primarily related to protection of the underlying epithelium have been attributed to it, as was reviewed by Neutra and Forstner (1987).

The major components of mucus are the high molecular weight glycoproteins that are characterized by a high carbohydrate content. A detailed summary of the structure and composition of glycoproteins was presented by Dekker (1990) and Neutra and Forstner (1987). The recovery and determination of the composition of crude mucus from ileal digesta collected from pigs was reported by Lien et al. (1997). In there study, N-acetylgalactosamine and N-acetylglucosamine were identified as potential markers for quantification of glycoproteins of gastric and intestinal origin, respectively. The glycoproteins contain two distinct protein regions. The "Native" region, representing over 95% of the glycoprotein (65% of the total protein) has a high concentration of serine, threonine and proline (40 to 70% of the protein of the native region). In this region oligosaccharide chains are attached to nearly every serine and threonine residue in the peptide core. The high proline content in this region has a role in maintaining the conformation of the protein core, allowing carbohydrate chains to be packed very closely. The tight packing of the oligosaccharides makes this region relatively resistant to proteolytic attack. The second region, based on its accessibility to proteolytic attack, is the nonglycosylated or "naked" region (35% of the total protein). The amino acid content in this region is similar to that of an average globular protein but has a high concentration of cysteine. This region has a role in the structure of mucus via the joining of the glycoprotein subunits by disulfide bridges.

There is limited information on the contribution of amino acids from small intestinal secretions to total endogenous secretions. Horszczaruk et al. (1974) estimated the daily secretion of intestinal nitrogen at 10 to 12 g using isolated gut loops in pigs. They calculated these values based on the assumption that the secretion rate in the isolated

gut segment reflected the total small intestinal secretion. However, Buraczewska (1979) showed that daily nitrogen secretion was 0.97 g/m in the proximal region of the small intestine and 0.48 g/m in the ileum. Total nitrogen secretion was estimated to be 14.4 g/day. Of this amount, 86 to 90% was soluble (50 to 70% in the form of amino acid nitrogen). The remaining soluble nitrogen was probably amines, amides, amino sugars and urea. Insoluble nitrogen consisted mainly of sloughed epithelial cells. The small intestinal nitrogen secretions reported by Buraczewska (1979) are minimum estimates because they were determined using "empty" isolated gut loops (i.e. in the absence of stimuli from dietary origin). Other studies, of a morphological nature, suggest that factors such as food intake and antinutritional factors may also affect small intestinal secretions (Li et al. 1990, 1991a, b; Kik, 1991). The effect of soybean trypsin inhibitors on the ileal recovery and amino acid composition of endogenous protein should be investigated.

Bacterial Contribution to Endogenous Losses

Recovery of nitrogen either as amino acids or nonprotein nitrogen at the terminal ileum represents a net loss to the pig. Dietary and endogenous protein or nonprotein nitrogen secreted into the lumen of the small intestine may be utilized by the enterobacteriaceae for synthesis of bacterial protein that is mainly unavailable for absorption by the pig. The proportion of bacterial protein produced from nonprotein nitrogen in the small intestine is probably dependent on dietary quantity and quality. Bacteria in the large intestine of pigs utilize nonprotein nitrogen for protein synthesis when provided with fermentable energy substrates. Diets with high levels of fermentable carbohydrates shift the excretion of nitrogen from urine to feces in pigs (Heijnen, 1997). On the other hand, small intestinal bacteria may preferentially utilize dietary and endogenous protein for growth or as energy substrates making it unavailable to the pig. Dugan et al. (1994) using a new and sensitive High Performance Liquid Chromatography technique to measure the bacterial marker diaminopimelic acid determined that about 30% of amino acids at the distal ileum of pigs were of bacterial origin. In this context, bacterial protein must necessarily form a substantial proportion of endogenous (nondietary) losses. Bacterial utilization of dietary and endogenous protein could shift the metabolic homeostasis of amino acids and, hence, requirements of the pig. In terms of protein metabolism of pigs, quantifying the contribution of amino acids from bacterial origin versus endogenous secretions is of importance. The effect of time after weaning on bacterial contributions to endogenous losses of nitrogen and amino acids in piglets can be estimated using diaminopimelic acid. Future diet

formulations could potentiate a synergism with small intestinal bacteria that would reduce the dietary protein requirements of pigs.

True ileal amino acid digestibilities

It is now generally accepted that ileal rather than fecal analysis is the preferred method for determining amino acid digestibilities in feedstuffs fed to pigs because it eliminates the modifying effect of bacterial metabolism in the large intestine (Zebrowska, 1973). The apparent ileal digestibility of amino acids in a number of different feedstuffs have been determined and are summarized in a number of reviews (Tanksley and Knabe, 1984; Sauer and Ozimek, 1986 and Sauer and de Lange, 1992). However, apparent digestibilities of amino acids based on recoveries at the distal ileum of pigs are not corrected for losses of amino acids from endogenous secretions. There are substantial losses of endogenous proteins, peptides and amino acids into the digestive tract in the form of sloughed epithelial cells, enzymes in saliva, bile, pancreatic and gastrointestinal secretions, plasma proteins and mucus (Souffrant, 1991). A number of different factors can also influence the loss of these endogenous proteins. Dietary level of intake (Souffrant, 1991), fiber source (Sauer et al. 1977) or antinutritional factors, such as soybean glycinin and β-conglycinin (Li et al. 1990; Li et al. 1991a, b), lectins (Kik, 1991; Schulze et al. 1995) and soybean trypsin inhibitors (Schulze, 1994) change apparent amino acid digestibilities among similar feedstuffs. Moreover, there is no information on losses of endogenous protein and ileal digestibilities of amino acids in piglets the first few weeks after weaning. The gastrointestinal tract of newly weaned piglets has to adapt to non-milk based diets before endogenous proteins that fully support digestion are secreted (Corring, 1980; Makkink and Verstegen, 1990). During this adaptation period there may be substantial losses of endogenous protein in piglets. To determine the "True" ileal digestibility (apparent values corrected for endogenous losses) of dietary amino acids a correction for endogenous losses has to be made. In this context, true ileal amino acid digestibilities were estimated in growing pigs fed soy flour with high or low concentration of soybean trypsin inhibitors and in newly weaned piglets fed protease-treated and untreated soybean meal. This information adds to our understanding of the dietary amino acid requirements of pigs.

Homoarginine Ratio Method

The true digestibility of dietary lysine (Hagemeister and Erbersdobler, 1985) and protein (Schmitz et al. 1991) have been determined using a recent novel direct approach known as the homoarginine technique. The technique involves the guanidination of dietary protein to convert lysine into the synthetic derivative homoarginine. The content of homoarginine in ileal digesta of pigs can then be measured as an indication of the true digestibility of dietary lysine based on the assumption that homoarginine is not incorporated into endogenous protein. It is assumed that homoarginine does not affect digestion or absorption of dietary protein and once absorbed is converted back to lysine and urea in the hepatic tissue. In this respect, the dietary requirement for lysine is at least partially fulfilled. However, a study using guanidinated lysine in three milk proteins, fed to minipigs, showed that digestibilities were reduced by 1.2 to 4.7 percentage units by the chemical transformation process (Drescher et al. 1994). Furthermore, prolonged feeding of guanidinated feed protein has been shown to cause detrimental changes in amino acid homeostasis in rats (Tews and Harper, 1986).

Marty et al. (1994) used the homoarginine technique and the reported composition of endogenous protein in pigs fed a nitrogen-free diet (de Lange et al. 1989) to indirectly estimate the recoveries of endogenous amino acids in pigs fed five different processed soybean products. They fed nitrogen-free meals before and after giving a single guanidinated test meal to maintain normal physiological function of the gastrointestinal tract during collection of digesta. However, comparison of apparent and true digestibilities, in their study, indicated incomplete clearance of amino acids from dietary meals given before the guanidinated protein test meal. Thereby, direct determination of endogenous recoveries of amino acids other than lysine was not possible. An alternative to their approach would be to fed meals of hydrolyzed casein, containing an equivalent level of crude protein, before and after the guanidinated protein test meals. Casein amino acids are completely digested (Chung and Baker, 1992).

The homoarginine technique was limited to direct estimates of endogenous lysine and nitrogen losses until Siriwan et al. (1994) reported recoveries of endogenous amino acids in poultry based on the ratio of homoarginine to amino acids in guanidinated test meals and digesta. Using an indigestible dietary marker the flow of endogenous amino acids at the distal ileum of pigs could be determined using the homoarginine ratio method. This method needs to be evaluated as a potential direct approach to estimating endogenous ileal amino acid recoveries in pigs fed different diets.

Gastrointestinal Exchange of Amino Acids

Measuring the exchange of amino acids across the gastrointestinal tract may be an alternative approach to measuring recoveries of endogenous amino acids. Blood plasma free amino acids have traditionally been considered the primary transport pool of blood. However, other blood pools, particularly plasma peptides and red blood cells have a dynamic role in the transport and exchange of amino acids throughout the animal body. Furthermore, the exchange of amino acids across the gastrointestinal tissues of pigs may not be the same for these blood pools. Koeln et al. (1993) reported differences between the different blood pools in the exchange of amino acid across the gastrointestinal tract and liver of calves. The exchange of amino acids from the gastrointestinal tract was also threefold greater in plasma peptides than that of the plasma free amino acids. They further suggested that the role of the blood transport pools may depend on species, diet and physiological state. There is limited information on exchange and transport of amino acids into and out of the plasma peptide and red blood cell pools of pigs. Estimating the proportion of amino acids from dietary and endogenous origin that are exchanged across the gastrointestinal tract into the plasma, plasma peptide and red blood cell pools should be determined. Differences in exchange between the blood pools could have important implications for future nutritional strategies to improve protein digestibility in pigs. Dietary protein digested and absorbed to a greater degree in peptide rather than free amino acid form may require less synthesis and secretion of endogenous protein and enzymes. In terms of improving energetic efficiency and maintaining protein homeostasis this would be advantageous to the pig. Growing pigs receiving an intraduodenal infusion of an enzymatic hydrolysate of milk protein, comprised primarily of small peptides, had greater and more rapid absorption of amino acids than pigs receiving an equivalent mixture of free amino acids (Rerat et al. 1988). In this respect, the difference in digestibility coefficients between protein sources may, in part, be dependent on the proportion of amino acids absorbed as peptides. The origin of amino acids in the plasma free amino acid, red blood cell and plasma peptide pools of pigs in a fed state should be estimated.

Thesis Outline

The primary objectives of this thesis were twofold. First, the homoarginine ratio method was evaluated as a technique for measuring ileal recovery of endogenous amino acid in pigs. In the context of understanding how soybean trypsin inhibitors affect

endogenous amino acid recoveries, the homoarginine ratio method was applied to growing pigs fed a soy flour with high or low concentration of soybean trypsin inhibitors [**Chapter 1**] and to newly weaned piglets fed protease-treated and untreated soybean meal [**Chapter 4**]. The efficacy of pretreating soybean meal with a commercial protease to increase protein solubility and reduce content of soybean trypsin inhibitors [**Chapter 2**] was assessed. In addition, the effect of feeding protease-treated soybean meal and time after weaning on apparent ileal amino acid digestibilities in newly weaned piglets [**Chapter 3**] were determined. Bacterial contribution to total and endogenous recoveries of amino acids in piglets soon after weaning was estimated using diaminopimelic acid as a bacterial marker [**Chapter 5**]. The second objective was to determine the importance of the exchange of amino acids across the portal vein-drained tissue into and out of the plasma and red blood cell free amino acid pools and the plasma peptide pool of pigs fed wheat gluten as protein source [**Chapter 6**]. The exchange of amino acids from dietary and endogenous origin into these blood transport pools was estimated. Finally, the main conclusions and implications for determining endogenous recoveries of amino acids were summarized in the **General Discussion**.

Literature Cited

Auclair, E. 1986. Etude des pertes azotees d'origine endogene dans le tube digestif chez trois especes monogastriques: le porc, le coq et le rat. PhD. Thesis. University of Clermont, France.

Bikker, P. 1994. Protein and lipid accretion in body components of growing pigs: effects of body weight and nutrient intake. PhD. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

Buraczewska, L. 1979. Secretion of nitrogenous compounds into the small intestine of pigs. Acta Physiol.Pol. 30: 319-326.

Chung, T.K., and D.H. Baker. 1992. Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. J. Anim. Sci. 70: 3781-3790.

Corring, T., and J. Jung. 1972. The amino acid composition of pig pancreatic juice. Nutr. Rep. Int. 6: 187-190.

Corring, T., A. Aumaitre, and A. Rerat. 1972. Fistulation permanente du pancreas exocrine chez le porc. Application: response de la secretion pancreatique au repas. Anal. Biol. Anim. Biochim. Biophys. 12: 109-124.

Corring, T. 1980. The adaptation of digestive enzymes to the diet: Its physiological significance. Reprod. Nutr. Develop. 20: 1217-1235.

Dekker, J. 1990. Gastric mucin, structure and biosynthesis. PhD. Thesis. Rijksuniversiteit Utrecht, Utrecht, The Netherlands.

Drescher, K., H. Hagemeister, M. de Vrese, N. Roos, K. Middendorf and P. Rantamaki. 1994. Guanidination (homoarginine-labeling) of proteins does affect prececal ^{15}N recovery in pigs. In: W.B. Souffrant and H. Hagemeister (Ed.) Proceedings of the VIth International Symposium on Digestive Physiology In Pigs. EAAP Publication No. 80. p 64-66. Bad Doberan, Germany.

Dugan, M.E.R., W.C. Sauer, J.M. Dugan Jr. and W.R. Caine. 1994. Determination of bacterial amino acid contribution to ileal digesta from pigs using ^{35}S and DAPA marker techniques. J. Anim. a. Feed Sci. 3: 149-159.

Gabert, V.M., W.C. Sauer, S. Li, and M.Z. Fan. 1996. Exocrine pancreatic secretions in young pigs fed diets containing fababeans (*Vicia faba*) and peas (*Pisum sativum*): concentrations and flows of total, protein-bound and free amino acids. J. Sci. Food Agric. 70: 256-262.

Hagemeister, H., and H.F. Erbersdobler. 1985. Chemical labelling of dietary protein by transformation of lysine to homoarginine: a new technique to follow intestinal digestion and absorption. Proc. Nutr. Soc. 44: 133A.

Hee, J.H., W.C. Sauer, R. Berzins, and L. Ozimek. 1985. Permanent re-entrant diversion of porcine pancreatic secretions. Can. J. Anim. Sci. 65: 451-457.

Hee, J.H., W.C. Sauer, and R. Mosenthin. 1988a. The measurement of pancreatic secretions in the pig with the pouch technique. J. Anim. Physiol. a. Anim. Nutr. 60: 241-248.

Hee, J.H., W.C. Sauer, and R. Mosenthin. 1988b. The effect of feeding on the pancreatic secretions in the pig. J. Anim. Physiol. a. Anim. Nutr. 60: 249-256.

Horszczaruk, F., L. Buraczewska, and S. Buraczewski. 1974. Amount and composition of intestinal juice collected from isolated intestinal loops of pigs. Rocz. Nauk Rolm. B-95: 69-77.

Heijnen, M.L. 1997. Physiological effects of consumption of resistant starch. PhD. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

Huisman, J., M.W.A. Verstegen, P. van Leeuwen, and S. Tamminga. 1993. Reduction of N pollution by decrease of the excretion of endogenous N in pigs. In: M.W.A. Verstegen, L.A. den Hartog, G.J.M. van Kempen and J.H.M. Metz (Ed.) Nitrogen Flow in Pig Production and Environmental Consequences. p 55-61. Pudoc Scientific Publishers, Wageningen.

Juste, C., T. Corring, and Y. Le Coz. 1983. Bile secretion in the fistulated pig: effect of the method used for bile reinfusion. Reprod. Nutr. Dev. 23: 765-773.

Kik, M.J.L. 1991. Effect of lectins in legume seeds on the structure and function of the small intestinal mucosa. PhD. Thesis. State University of Utrecht, The Netherlands.

Koeln, L.L., T.G. Schlagheck, and K.E. Webb, Jr. 1993. Amino acid flux across the gastrointestinal tract and liver of calves. J. Dairy Sci. 76: 2275-2285.

de Lange, C.F.M., W.C. Sauer, and W.B. Souffrant. 1989. The effect of 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. 67: 755-762.

Li, D.F., J.L. Nelssen, P.G. Reddy, F. Blecha, J.D. Hancock, G.L. Allee, R.D. Goodband, and R.D. Klemm. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. J. Anim. Sci. 68: 1790-1799.

Li, D.F., J.L. Nelssen, P.G. Reddy, F. Blecha, R.D. Klemm, D.W. Giesting, J.D. Hancock, G.L. Allee, and R.D. Goodband. 1991a. Measuring suitability of soybean products for early-weaned pigs with immunological criteria. J. Anim. Sci. 69: 3299-3307.

Li, D.F., J.L. Nelssen, P.G. Reddy, F. Blecha, R.D. Klemm, and R.D. Goodband. 1991b. Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci. 69: 4062-4069.

Li, S., W.C. Sauer, S. Huang, and R.T. Hardin. 1997. Response of pancreatic secretions to feeding diets with low and high levels of soybean trypsin inhibitors in growing pigs. J. Sci. Food Agric. (in press).

Lien, K.A., W.C. Sauer, and M. Fenton. 1997. Recovery and composition of crude mucus in ileal digesta of pigs fed a protein-free diet. Z. Ernahrungswiss 36: 182-190.

Marty, B.J., E.R. Chavez, and C.F.M. de Lange. 1994. Recovery of amino acids at the distal ileum for determining apparent and true ileal amino acid digestibilities in growing pigs fed various heat-processed full-fat soybean products. J. Anim. Sci. 72: 2029-2037.

Makkink, C.A., and M.W.A. Verstegen. 1990. Pancreatic secretions in pigs. J. Anim. Physiol. Anim. Nutr. 64: 190-208.

Mosenthin, R., W.C. Sauer, and C.F.M. de Lange. 1992. Tracer studies of urea kinetics in growing pigs: I. The effect of intravenous infusion of urea on urea recycling and the site of urea secretion into the gastrointestinal tract. J. Anim. Sci. 70: 3458-3466.

Neutra, M.R., and J.F. Forstner. 1987. In: Physiology of the Gastrointestinal Tract (2nd Ed.). pp 975-1009. Raven Press, New York.

Rerat, A., C. Simoes Nunes, F. Mendy, and L. Roger. 1988. Amino acid absorption and production of pancreatic hormones in non-anaesthetized pigs after duodenal infusions of a milk enzymic hydrolysate or free amino acids. Br. J. Nutr., 60, 121-136.

Sauer, W.C., and C.F.M. de Lange. 1992. Novel methods for determining protein and amino acid digestibilities in feedstuffs. In: S. Nissen (Ed.) Modern Methods in Protein Nutrition and Metabolism. p 87-120. Academic Press Inc., San Diego, USA.

Sauer, W.C., and L. Ozimek. 1986. Digestibility of amino acids in swine: results and their practical application. A review. Livest. Prod. Sci. 15: 367-388.

Sauer, W.C., S.C. Stothers and R.J. Parker. 1977. Apparent and true availablity of amino acids in wheat and milling by-products for growing pigs. Can. J. Anim. Sci. 57: 775-784.

Schmitz, M., H. Hagemeister, and H.F. Erbersdobler. 1991. Homoarginine labeling is suitable for determination of protein absorption in miniature pigs. J. Nutr. 121: 1575-1580.

Schulze, H. 1994. Endogenous ileal nitrogen losses in pigs: dietary factors. PhD. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

Schulze, H., H.P. Saini, J. Huisman, M. Hessing, W. van den Berg, and M.W.A. Verstegen. 1995. Increased nitrogen secretion by inclusion of soya lectin in the diets of pigs. J. Sci. Food. Agric. 69: 501-510.

Siriwan, P., W.L. Bryden, and E.F. Annison. 1994. Use of guanidinated dietary protein to measure losses of endogenous amino acids in poultry. Br. J. Nutr. 71: 515-529.

Souffrant, W.C. 1991. Endogenous nitrogen losses during digestion in pigs. In: M.W.A. Verstegen, J. Huisman and L.A. den Hartog (Ed.) Proceedings of the Vth International Symposium on Digestive Physiology In Pigs. EAAP Publication No. 54. p 147-166. PUDOC, Wageningen (Doorwerth), The Netherlands.

Tanksley, T.D., Jr., 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. p 75. Butterworths, London.

Tews, J.K., and A.E. Harper. 1986. Induction in rats of lysine imbalance by dietary homoarginine. J. Nutr. 116: 1910-1921.

Zebrowska, T. 1973. Digestion and absorption of nitrogenous compounds in the large intestine of pigs. Roczn. Nauk Roln. 95B (3): 85-90.

Zebrowska, T., A.G. Low, and H. Zebrowska. 1983. Studies on gastric digestion of protein and carbohydrates, gastric secretion and exocrine pancreatic secretion in the growing pig. Br.J.Nutr. 49: 401-410.

Zebrowska, T., and A.G. Low. 1987. The influence of diets based on whole wheat, wheat flour and wheat bran on exocrine pancreatic secretion in pigs. J.Nutr. 117: 1212-1216.

1 Guanidinated Protein Test Meals With Higher Concentration of Soybean Trypsin Inhibitors Increase Ileal Recoveries of Endogenous Amino Acids In Pigs

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Guanidinated Protein Test Meals With Higher Concentration of Soybean Trypsin Inhibitors Increase Ileal Recoveries of Endogenous Amino Acids In Pigs

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ABSTRACT

The amino acid concentrations of cornstarch-based guanidinated unprocessed (UGM) and autoclaved (AGM) Nutrisoy (defatted soy flour) protein test meals were compared to the respective unguanidinated Nutrisoy diets. Endogenous ileal recoveries and true digestibilities of amino acids were determined in six growing pigs, fitted with a simple T-cannula at the distal ileum, fed the guanidinated protein test meals. The UGM and AGM contained 13.4 (high) and 3.0 (low) g/kg dry matter of soybean trypsin inhibitors (SBTI), respectively. The experiment was a two period cross-over design with each period lasting 15 d. On d 14 of each period the pigs were fed the guanidinated test meals followed by 24 h continuous collection of digesta. Concentrations of crude protein and most of the amino acids in the test meals were higher than in the respective diets. Apparent ileal amino acid digestibilities of the test meals were similar ($P > 0.05$) to reported values for the respective diets and higher ($P < 0.05$) by 22.7 (cysteine) to 61.3 (tyrosine) percentage units for AGM compared to UGM. The ileal recoveries of endogenous amino acids in AGM-fed pigs were lower ($P < 0.05$) than UGM-fed pigs. Values ranged from -0.10 (arginine) to 0.64 (aspartate + asparagine) and from 0.84 (histidine) to 2.61 (tyrosine) g/kg dry matter intake for AGM- and UGM-fed pigs, respectively. True ileal amino acid digestibilities for AGM were higher ($P < 0.05$) than UGM with differences ranging from 12.7 (tyrosine) to 38.3 (leucine) percentage units. In conclusion, ileal recoveries of endogenous amino acids were increased in pigs fed guanidinated protein test meals with the higher concentration of SBTI.

Key Words: Pigs, Endogenous amino acids, Soybean trypsin inhibitors, Homoarginine

Introduction

Different methods for estimating endogenous ileal recoveries of amino acids in pigs have been reported in the literature. These methods include feeding protein-free diets (e.g. de Lange et al. 1989), peptide alimentation and synthetic amino acid diets (Butts et al. 1993), regression analyses (Furuya and Kaji 1986), ^{15}N-leucine, ^{15}N-isoleucine and ^{15}N-isotope dilution techniques (de Lange et al. 1992). The homoarginine technique described by Hagemeister and Erbersdobler (1985) has been used to estimate the ileal recovery of endogenous lysine in rats (Moughan and Rutherfurd, 1990) and pigs (Marty et al. 1994) and endogenous nitrogen in pigs (Schmitz et al. 1991, Barth et al. 1993). Marty et al. (1994) indirectly estimated the flow of endogenous amino acids based on their concentration relative to lysine in endogenous protein collected from pigs fed a protein-free diet as reported by de Lange et al. (1989). The homoarginine technique involves guanidination of dietary protein to chemically convert

lysine into the synthetic derivative homoarginine. The guanidinated protein is then fed as test meals. The technique has been used in a number of studies based on the assumption that the chemical transformation does not affect the digestion or absorption of the dietary protein. However, there are no reported comparisons of the amino acid composition of dietary protein before and after guanidination.

Addition of antinutritional factors such as Kunitz trypsin inhibitors (Barth et al. 1993) and lectins (Schulze et al. 1995) to diets increase the amount of undigested endogenous nitrogen leaving the small intestine of pigs. Barth et al. (1993) concluded that the amino acid composition of endogenous ileal nitrogen may be important in terms of maintaining protein homeostasis of pigs fed diets containing protease inhibitors. In this context, Siriwan et al. (1994) adapted the homoarginine technique to estimate recoveries of endogenous amino acids in poultry by determining changes in the ratio of homoarginine to amino acids in diet and digesta after feeding test meals of guanidinated casein and soybean protein. This homoarginine ratios method has not been used in studies with mammals and may be a relatively simple direct approach to determine ileal recoveries of endogenous amino acids.

The objectives of this study were twofold. Firstly, to compare the amino acid composition of guanidinated Nutrisoy (defatted soy flour) protein test meals and the respective unguanidinated Nutrisoy diets. Secondly, to measure endogenous ileal recoveries and true digestibilities of amino acids in growing pigs fed guanidinated Nutrisoy protein test meals with high or low concentration of soybean trypsin inhibitors (SBTI) using the homoarginine ratio method.

Experimental

Animals and diets. This study was carried out in conjunction with an experiment to determine fecal and ileal apparent digestibilities of energy and amino acids in pigs fed cornstarch-based diets with either unprocessed or autoclaved Nutrisoy (supplied by Archer Daniels Midlands Company, Decatur, IL; 530 g crude protein/kg dry matter) as the protein source (Li et al. 1997b). Details of animals and management are described in detail by Li et al. (1997b).

The experiment was carried out according to a two period crossover design (Petersen 1985). Six barrows, average body weight 53.3 ± 3.7 kg, fitted with a simple T-cannula at the distal ileum were housed in individual metabolism crates in a temperature-controlled (25 ± 1°C) room at the University of Alberta Metabolic Research Facility. Formulation of the experimental diets and guanidinated protein test meals are

Table 1.
Formulation of the experimental diets and guanidinated Nutrisoy[1] test meals and casein enzymatic hydrolysate (CEH) meals.

	Nutrisoy		CEH meals
Ingredient	Unprocessed	Autoclaved	
	g/kg; air dry basis		
Cornstarch	400.0	400.0	590.0
Unprocessed Nutrisoy	350.0	-	-
Autoclaved Nutrisoy	-	350.0	-
CEH[2]	-	-	223.0
Canola oil	50.0	50.0	50.0
Dextrose	100.0	100.0	100.0
Solka-Floc[3]	60.0	60.0	-
Biophos[4]	16.0	16.0	16.0
Mineral-vitamin premix[5]	10.0	10.0	10.0
Calcium carbonate[6]	5.0	5.0	5.0
Iodized salt[7]	3.0	3.0	3.0
Chromic oxide[8]	3.0	3.0	-
Antibiotics[9]	1.5	1.5	1.5
DL-Methionine	1.5	1.5	1.5

[1] Defatted soy flour. Supplied by Archer Daniels Midlands Company (MAD), Decatur, IL.

[2] Casein Enzymatic Hydrolysate. Supplied by Sigma Chemical Company, St. Louis, MO, USA.

[3] Solka-Floc, powdered cellulose. Supplied by James River Corp., Berlin, NH.

[4] Supplied by Continental Lime Ltd., Exshaw, AB. Provided the following (g/kg): 150 to 180 of available P and 240 of Ca.

[5] Supplied by Hoffmann-LaRoche Ltd., Meadowpine Bvd., Mississauga, ON, Canada. Provided the following (mg/kg diet): Retinyl palmitate, 2.27; cholecalciferol, 0.0125; DL-α-tocopheryl acetate, 40.0; menadione, 2.0; cyanocobalamin, 0.03; riboflavin, 12.0; niacin, 40.0; dl-pantothenate, 25.0; choline, 600.0; d-biotin, 0.25; folacin, 1.6; thiamin, 3.0; ethoxyquin, 5.0; Fe, 150.0; Mn, 20.0; Zn, 120.0; Cu, 125.0; I, 0.2; Se, 0.3.

[6] Supplied by Continental Lime Ltd., Exshaw, AB. Provided 380 g/kg of Ca.

[7] Supplied by Sifto Canada Inc., Mississauga, ON. Provided the following (g/kg): 990 of NaCl and 1.5 of I.

[8] Dysprosium chloride (0.116 g/kg of diet) included along with chromic oxide in the guanidinated protein test meals to provided 50 mg/kg of dysprosium.

[9] Veterinary LS-20 premix; supplied by The Upjohn Company, Animal Health Division, Orangeville, ON, Canada. Provided the following (g/kg premix): Lincomycin hydrochloride, 22; Spectinomycin sulphate, 22; mineral oil USP, 10; soybean meal carrier, 946.

presented in **Table 1**. The diets were formulated with either unprocessed or autoclaved Nutrisoy to contain 200 g crude protein/kg with 13.4 (high) and 3.0 (low) g SBTI/kg dry matter, respectively. The guanidinated protein test meals were formulated the same as the respective diets except that dysprosium chloride was included at 0.116 g/kg (providing approximately 50 mg/kg of dysprosium) as an additional digestibility marker specific to the test meals. Canola oil was included to meet NRC (1988) standards for digestible energy. Vitamins, minerals and DL-methionine were also included to meet or exceed NRC (1988) standards. Chromic oxide was included at 3 g/kg of diet as a digestibility marker.

The pigs were fed at a daily rate of 4 g/100 g body weight in two equal meals at 0800 and 2000 h throughout the experiment. The pigs were weighed at the beginning of each experimental period and their feed intake adjusted accordingly. Each period lasted 15 d. From d 1 to 13, the pigs were fed the experimental diets. At 0800 h on d 14 unprocessed (UGM) and autoclaved (AGM) Nutrisoy guanidinated protein test meals were offered to the pigs. A casein enzymatic hydrolysate (CEH) meal of 900 g was given before and after the guanidinated protein test meals (2000 h on d 13 and 14, respectively) to clear the small intestine of any undigested protein and chromic oxide originating from the previous unguanidinated Nutrisoy diets. As for the Nutrisoy diets, the CEH meals were formulated to contain 200 g crude protein/kg (Table 1). The amino acids in the CEH meals were assumed to be completely absorbed based on true digestibilities of cornstarch-based casein diets fed to pigs (Chung and Baker, 1992). Particular care was taken to ensure that the guanidinated test meals were completely consumed by the pigs by cleaning the feeders and crates prior to the test meal and suspending plastic sheets below the metabolism crates to collect spilled feed which was added back to the feeders. The guanidinated test meals were consumed within 2 h after being offered. Ileal digesta was collected continuously for 24 h starting immediately after the test meal was offered. The digesta was collected into a plastic bag which was connected to the barrel of the cannula of each pig. The bags contained 10 mL of formic acid (2.5 mol/L) to stop microbial activity. Bags were changed at least once every h and digesta were pooled within pig and period and frozen at -20°C until analyses.

The experimental proposal and surgical procedures were approved by the Animal Care Committees of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta and the Wageningen Institute of Animal Sciences at the Wageningen Agricultural University. The pigs were cared for in accordance with the guidelines established by CCAC (1980).

Preparation of the guanidinated test meals. Lysine residues in batches of unprocessed and autoclaved Nutrisoy were guanidinated as described by Schmitz et al. (1991). Batches (377 g) of the respective Nutrisoy were mixed with 1 L of deionized distilled water in 4 L beakers. One L of 0.4 mol/L methylisourea was added to each beaker and the pH adjusted with 4 mol/L NaOH to 10.3 which is optimal for guanidination of soybean protein according to Maga (1981). The methylisourea was previously prepared by dissolving and mixing a solution of 0.4 mol/L o-methylisourea hydrogen sulphate and 0.4 mol/L barium hydroxide octahydrate (Sigma Chemical, St. Louis, MO). The solution was then centrifuged and filtered to remove the barium sulphate precipitate. The Nutrisoy slurries were continuously stirred for 1 h and the beakers then covered with aluminum foil and stored in a cold room at 4°C for 4 d. The Nutrisoy slurries in the beakers were stirred each d and the pH adjusted to 10.3. The guanidination reaction was stopped by precipitation of the protein at pH 4.5 (isoelectric point) using 1 mol/L HCl (Schmitz et al. 1991). The contents in each beaker were transferred to four 1 L Beckman polypropylene containers and centrifuged for 10 min at 4,000 × g at 4°C. The supernatant was decanted leaving the guanidinated Nutrisoy. The Nutrisoy was washed three times to remove the excess methylisourea by resuspension in distilled water (pH adjusted to 4.5) and centrifuged and decanted, as previously described. The guanidinated Nutrisoy samples were transferred to stainless steel trays, frozen at -20°C, and freeze-dried. After drying, the batches of Nutrisoy were weighed to determine the dry matter recoveries. The resultant guanidinated material was crushed to a particle size similar to the original Nutrisoy.

Chemical analyses. Samples of the diets, guanidinated test meals and digesta were freeze-dried and ground through a 0.5-mm mesh screen in a Wiley mill (Arther H. Thomas Co., Philadelphia, PA) before analyses. Dry matter contents were determined according to AOAC (1990). The gross energy content of the diets and test meals were determined using a Parr 1241 Adiabatic Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, IL). The nitrogen contents were measured with an Automated N Analyzer (FP-428 Nitrogen Determinator, Leco® Corporation, St. Joseph, MI). Analyses of chromic oxide and dysprosium in the guanidinated test meals and ileal digesta were performed by Instrument Neutron Activation Analysis according to the procedure described by Kennelly et al. (1980). Samples of the test meals and digesta, approximately 5 g, were packed into 5 mL irradiation vials and irradiated at the University of Alberta SLOWPOKE II nuclear reactor. Following a suitable decay period, samples were counted by measuring the 320.1 and 108.2 keV γ-rays emitted by the radionuclides ^{51}Cr ($T^{1/2}$ = 27.7 d) and ^{165m}Dy ($T^{1/2}$ = 1.258 min.), respectively.

Analyses of amino acids in hydrolysates of the test meals and digesta were performed by weighing approximately 100 mg of sample into 13 × 100 mm screw-capped culture tubes and adding 3 mL of 6 mol/L HCl. The tubes were purged with nitrogen before sealing the screw cap and then incubated in an oven at 100°C for 24 h. Contents of amino acids were determined in duplicate aliquots of the hydrolysates by a fluorometric method involving pre-column derivatization with *o*-phthaldialdehyde and analysis by HPLC according to Jones and Gilligan (1983) using a Varian 5000 HPLC system with a Varian 9090 autosampler and a Varian Fluorichrom detector (excitation 340 nm, emission 450 nm; Varian Canada Inc., Mississauga, ON). The aliquots were injected on a Supelcosil 3 micron LC-18 reverse phase column (4.6 × 150 mm; Supelco, Sigma-Aldrich Canada Ltd., Mississauga, ON) equipped with a Supelco LC-18 reverse phase 20 to 40 micron guard column (4.6 × 50 mm). The run time for each sample was 38 min with homoarginine eluting off the column at about 22 min, just before alanine and tyrosine. Methionine and cysteine were determined as methionine sulphone and cysteic acid, respectively, after oxidation with performic acid according to AOAC (1990). The oxidized samples were dried and then hydrolyzed and analyzed as described for the other amino acids. Peaks for amino acids were recorded and integrated with the EZchrom™ Chromatography Data System (version 2.12, Shimadzu Scientific Instruments Inc., Columbia, MD).

The conversion of lysine to homoarginine (homo) in the guanidinated test meals was calculated as described by Rutherfurd and Moughan (1990), as follows:

$$\textbf{Conversion (g/100 g)} = [\textbf{homo}_{\text{Nutrisoy}}/(\textbf{lysine} + \textbf{homo})_{\text{Nutrisoy}}] \times 100. \quad \text{Eq. 1}$$

Calculation of flows and digestibilities of amino acids. The total flow of each amino acid (AA, g/kg dry matter intake) at the distal ileum was calculated using the respective amino acid (aa) concentration and chromic oxide or dysprosium as the indigestible markers (IDM), as follows:

$$(\text{AA})_{\text{flow}} = (\text{aa})_{\text{digesta}} \times [\text{IDM}_{\text{diet}} / \text{IDM}_{\text{digesta}}]. \quad \text{Eq. 2}$$

The endogenous recovery of each amino acid (EndAA) at the distal ileum was calculated from the ratio of homoarginine (homo) to the respective amino acid concentrations in the guanidinated test meals and ileal digesta as suggested by Siriwan et al. (1994), as follows:

$$(\text{EndAA}) = [(\text{homo})_{\text{diet}} / (\text{aa})_{\text{diet}}] - [(\text{homo})_{\text{digesta}} / (\text{aa})_{\text{digesta}}] \times (\text{AA})_{\text{flow}}. \quad \text{Eq. 3}$$

The exogenous (dietary) recovery of each amino acid (ExoAA) at the distal ileum was calculated as follows:

$$(ExoAA) = (AA)_{flow} - (EndAA). \quad \text{Eq. 4}$$

Apparent and true ileal digestibilities were expressed as percentages. Apparent digestibilities (AD) of crude protein and amino acids (including homoarginine) were calculated as follows:

$$AD_{aa} = [(aa)_{diet} - (AA)_{flow} / (aa)_{diet}] \times 100. \quad \text{Eq. 5}$$

The apparent digestibility of homoarginine is assumed to be similar to the true digestibility of lysine as proposed by Hagemeister and Erbersdobler (1985). True ileal digestibility (TD) of each amino acid was calculated from their respective concentration in ileal digesta as follows:

$$TD_{aa} = [(aa)_{diet} - [(AA)_{flow} - (EndAA)]] / (aa)_{diet}] \times 100. \quad \text{Eq. 6}$$

Estimates of total flow, endogenous and exogenous recoveries and apparent and true digestibilities of lysine were determined using the total of residual lysine plus homoarginine in the test meals and digesta.

Statistical analysis. The results were subjected to analysis of variance using the General Linear Model Procedure of the SAS Institute Inc., (1990). The statistical model included experimental periods (P) and dietary treatments (D) as main effects and their interaction with pigs within group as the source of variation:

$$Y_{ij} = \mu + P_i + D_j + P \times D + \epsilon_{ij}$$

where i = 2 and j = 2. Treatment means were compared using the Students' *t*-test according to Steel and Torrie (1980). Means were considered to be different when the *P* value was less than 0.05.

Results and Discussion

The crude protein and amino acid contents of the Nutrisoy diets and corresponding guanidinated protein test meals are presented in **Table 2**. Recoveries of dry matter after guanidination of the Nutrisoy batches used for the preparation of UGM and AGM were 620 and 760 g/kg, respectively. The apparent loss of material from the Nutrisoy batches were assumed to consist mainly of soluble carbohydrates and some amino acids, lost during the multiple washing to remove methylisourea after

Table 2.
Chemical analyses and amino acid content of the unprocessed and autoclaved Nutrisoy diets (UND and AND, respectively) and guanidinated test meals (UGM and AGM, respectively) and the casein enzymatic hydrolysate (CEH) meals.

	Nutrisoy Diets		Guanidinated Test Meals		CEH Meals
Item	UND	AND	UGM	AGM	
	g/kg dry matter; unless specified				
Dry matter	919	917	926	911	938
Crude protein	203	207	254	239	194
GE[1] (MJ/kg)	18.8	18.8	18.2	18.4	17.7
SBTI[2]	3.3	3.3	-	-	-
Amino acids					
Homoarginine	0	0	6.0	3.2	0
Indispensable					
Arginine	13.8	13.9	13.7	13.0	5.6
Histidine	5.0	5.0	5.5	5.3	5.0
Isoleucine	9.1	9.4	10.1	10.9	10.3
Leucine	14.9	15.5	17.2	18.2	17.6
Lysine	12.1	11.7	7.0	7.5	14.5
Methionine	7.7	7.7	7.7	8.2	10.9
Phenylalanine	10.0	10.4	10.4	11.0	8.7
Threonine	7.2	7.3	7.7	8.2	7.2
Valine	9.1	9.5	10.9	11.7	12.8
Dispensable					
Alanine	8.5	8.6	10.0	10.4	6.1
Aspartate + Asparagine	19.6	19.7	25.1	26.0	14.0
Cysteine	10.0	8.6	10.0	8.7	1.8
Glutamate + Glutamine	34.9	36.2	39.5	40.6	42.5
Glycine	8.4	8.6	9.7	9.3	3.5
Serine	9.4	9.6	10.4	10.7	9.7
Tyrosine	5.2	5.5	5.2	5.7	3.3

[1] GE; Gross Energy.

[2] SBTI; Soybean Trypsin inhibitors.

guanidination. As the protein test meals were formulated using the same quantities of guanidinated Nutrisoy as with the diets this resulted in an increase in their content of crude protein and most of the amino acids. The crude protein contents of UGM and AGM were 25 and 15 g/100 g higher, respectively, than in the unprocessed and autoclaved Nutrisoy diets. The total sum of the amino acids presented in Table 2 accounted for 91 and 90 g/100 g of the crude protein content of the unprocessed and autoclaved Nutrisoy diets, respectively. Corresponding values were 81 and 87 g/100 g for UGM and AGM. These differences suggest that methylisourea may not have been entirely removed by washing the batches of guanidinated Nutrisoy, resulting in non-amino acid nitrogen enrichment in the test meals. However, the indispensable to dispensable amino acid ratio in the diets was 0.93 and higher than values of 0.88 and 0.87 for UGM and AGM, respectively. In this respect, the concentration of most of the dispensable amino acids, particularly aspartate + asparagine and glutamate + glutamine were increased in the protein test meals. On the other hand, the concentration of the indispensable amino acids were usually similar between the protein test meals and the respective diets. This suggests a disproportionate loss of more soluble indispensable than dispensable amino acids from the batches of guanidinated Nutrisoy. Differences in the proportion of amino acids lost after guanidination was probably related to their content in the different protein components of plant material.

The conversions of lysine to homoarginine according to equation 1 were 46 and 30 g/100 g for UGM and AGM , respectively. The sum of the concentrations of homoarginine and residual lysine of UGM was 0.9 g/kg higher than the lysine concentration of the unprocessed Nutrisoy diet. Whereas, the sum of the concentrations of homoarginine and residual lysine of AGM was 1.0 g/kg lower than the lysine concentration of the autoclaved Nutrisoy diet. The concentration of arginine was also 0.9 g/kg lower in AGM compared to the respective diet. The lower concentration of residual lysine plus homoarginine in AGM compared to the lysine concentration of the autoclaved Nutrisoy is difficult to explain. Perhaps, autoclaving the Nutrisoy before guanidination may have denatured the protein in a manner that caused a disproportionate loss of lysine and arginine during washing. The Nutrisoy was autoclaved using steam at 120°C, at 32 pounds per square inch, for 15 min. With the exception of lower content of lysine and cysteine these conditions caused only minor increases in the amino acid concentration of autoclaved compared to unprocessed Nutrisoy in the diets. Nevertheless, decrease in the content of lysine in the autoclaved Nutrisoy indicates temperature sensitivity that would, in part, explain the lower concentration of homoarginine and residual lysine in AGM.

Hagemeister and Erbersdobler (1985) first proposed guanidination of protein to convert dietary lysine to the amino acid derivative homoarginine to directly quantify the ileal recovery of endogenous lysine in pigs. The use of guanidinated test meals involves several assumptions which have been discussed elsewhere (e.g., Moughan and Rutherfurd 1990, Schmitz et al. 1991, Marty et al. 1994). The determination of endogenous amino acid recoveries using the homoarginine ratio method assumes that guanidination does not affect the digestion and absorption of the test protein and that the absorption of homoarginine is similar to lysine. It is also assumed that homoarginine is not incorporated into endogenous secretions. However, guanidinated protein test meals have usually been fed to rats (Moughan and Rutherfurd 1990), poultry (Siriwan et al. 1994) and small pigs (e.g., Schmitz et al. 1991, Butts et al. 1993, Barth et al. 1993) that require only small quantities of guanidinated protein. Rutherfurd and Moughan (1990) used dialysis against distilled water instead of multiple washing and centrifugation to remove excess methylisourea. This approach is suitable for preparation of small but not for larger quantities of guanidinated protein. Imbeah et al. (1996) determined the optimum conditions for guanidination of casein and soybean protein and reported a maximum conversion of 78 g/100 g in soybean protein (incubated in 0.4 mol/L methylisourea solution, at pH 10.5 and 20°C for 24 h) when the molar ratio of lysine to methylisourea was 1:10. In the present study, batches of Nutrisoy were guanidinated with a 1:6 ratio of lysine to methylisourea in 0.2 mol/L methylisourea solution (1 L of distilled water plus 1 L of 0.4 mol/L of methylisourea solution), at pH 10.3 and 4°C for 96 h. Under these conditions the conversion of lysine residues to homoarginine was lower than reported by Imbeah et al. (1996). Interestingly, Imbeah et al. (1996) also reported 8 to 11 g/100 g higher conversion rates in large- (5 kg) compared to laboratory-scale (20 g) batches of soybean protein. They did not explain these differences in conversion but it is likely, as seems to be the case in the present study, that soluble protein and carbohydrate were removed from soybean protein during the multiple washing to remove methylisourea. A proportionally greater loss of lysine than homoarginine residues during the procedure for guanidination of the large batches of soybean protein would account for their greater conversion rate. An alternative method of guanidinating large quantities of protein needs to be developed for studies with larger animals.

A large proportion of the lysine residues in the test protein need to be guanidinated for random distribution of homoarginine (e.g., Moughan and Rutherfurd 1990, Siriwan et al. 1994). Whether the conversion of lysine to homoarginine occurred in a uniform manner with respect to the composition of the guanidinated Nutrisoy

protein is difficult to measure. Siriwan et al. (1994) assumed random distribution of homoarginine residues in casein and soybean protein based on a constant ratio between homoarginine and the other amino acids following sequential *in vitro* enzymatic

Table 3.
Endogenous and exogenous (dietary) recoveries of amino acids in growing pigs fed unprocessed (UGM) and autoclaved (AGM) Nutrisoy guanidinated test meals.

	Endogenous[1]			Exogenous[1]		
Item	UGM	AGM	SEM[2]	UGM	AGM	SEM[2]
	g/kg dry matter intake					
Sum of Amino acids	24.80	4.00	3.75	93.91	42.04	13.34
Indispensable						
Arginine	0.97	-0.10	0.05	5.70	1.43	0.56
Histidine	0.84	0.10	0.09	1.69	0.80	0.14
Isoleucine	1.85	0.08	0.08	4.69	1.64	0.41
Leucine	1.70	0.00	0.08	8.84	2.44	0.76
Lysine[3]	0.86	0.44	0.09	5.31	2.38	0.52
Methionine	1.35	0.36	0.11	3.09	1.74	0.21
Phenylalanine	1.54	-0.07	0.05	4.55	1.30	0.41
Threonine	2.08	0.50	0.15	3.14	1.91	0.30
Valine	1.73	0.14	0.09	4.95	1.94	0.41
Dispensable						
Alanine	1.43	0.23	0.08	4.14	1.91	0:37
Aspartate + Asparagine	1.52	0.64	0.03	13.03	8.02	0.80
Cysteine	1.07	0.46	0.05	3.78	1.82	0.25
Glutamate + Glutamine	1.37	0.35	0.05	20.42	9.47	1.21
Glycine	1.90	0.55	0.15	4.43	2.25	0.43
Serine	1.98	0.30	0.12	4.85	2.19	0.42
Tyrosine	2.61	0.02	0.27	1.30	0.80	0.17

[1] Means in the same row, within endogenous or exogenous recoveries of amino acids were always different ($P < 0.05$).

[2] Standard error of the mean (n = 6).

[3] Endogenous recoveries of lysine for UGM and AGM, calculated as the ratio of homoarginine to residual lysine plus homoarginine in the test meals and digesta. Endogenous and exogenous recoveries of only residual unguanidinated lysine in UGM and AGM were 2.51 and 0.82 g/kg dry matter intake (SEM; 0.41) and 3.66 and 2.00 g/kg dry matter intake (SEM; 0.17), respectively.

digestion. However, Schmitz et al. (1991) reported that the *in vitro* rate of proteolysis of guanidinated casein by trypsin was slower ($P < 0.05$) than for unguanidinated casein. Whereas, the rate of *in vitro* proteolysis by chymotrypsin was not affected ($P > 0.05$) by guanidination. This suggests that replacing lysine with homoarginine in guanidinated protein may actually impede enzymatic digestion. Consequently, constant ratios of homoarginine to amino acids following sequential enzymatic digestion would not necessarily indicate a random distribution of the lysine derivative.

Endogenous amino acid recoveries were higher ($P < 0.05$) in pigs fed UGM (24.80 g/kg dry matter intake) than AGM (4.00 g/kg dry matter intake) with all individual amino acids following a similar pattern (**Table 3**). The endogenous amino acid recoveries ranged from 0.84 (histidine) to 2.61 (tyrosine) g/kg dry matter intake for UGM and from -0.10 (arginine) to 0.64 (aspartate + asparagine) g/kg dry matter intake for AGM. Differences in endogenous recoveries between UGM and AGM were greatest for the aromatic, branched-chain and hydroxy amino acids. The exogenous (dietary) recoveries of all amino acids were higher ($P < 0.05$) in pigs fed UGM compared with AGM (Table 3). The exogenous recoveries ranged from 1.30 (tyrosine) to 20.42 (glutamate + glutamine) g/kg dry matter intake for UGM and from 0.80 (histidine and tyrosine) to 9.47 (glutamate + glutamine) g/kg dry matter intake for AGM.

In the present study, it was intended that flows of amino acids would be determined using dysprosium as a specific marker in the guanidinated protein test meals. However, flows of most of the amino acids in the UGM-fed pigs, based on dysprosium (data not shown), were higher than estimates based on chromic oxide. In the AGM-fed pigs flows of amino acids determined using dysprosium were not different ($P > 0.05$) from estimates based on chromic oxide. These results suggest an incomplete recovery of dysprosium from the test meal in UGM-fed pigs. Imbeah et al. (1995) discussed the possibility of dysprosium migrating between particulate matter or forming complexes with endogenous organic compounds in the digestive tract of pigs, thus affecting estimates of marker passage.

The proportion of total amino acids, from either endogenous or exogenous origin, recovered at the distal ileum of pigs, depends on the true digestibility of the protein source being investigated and the content of antinutritional factors such as SBTI. Previous studies have reported greater increases in endogenous than exogenous nitrogen recoveries in pigs fed diets with higher levels of SBTI. For instance, Barth et al. (1993) found that exogenous nitrogen represented only 7.9 and 9.2 g/100 g of total nitrogen recovered at the distal ileum of miniature pigs fed guanidinated casein meals

supplemented without or with 3.0 g of purified Kunitz trypsin inhibitors, respectively. This relatively small change in exogenous nitrogen recovery is surprising given the high rate of formation of trypsin inhibitor-enzyme complexes that would be expected with the level of Kunitz trypsin inhibitors supplemented to the diet. In the present study, endogenous and exogenous nitrogen recoveries, calculated from the respective sum of amino acids, assuming 16 g nitrogen/ 100 g protein, were 22 and 78 g/100 g, respectively, for UGM-fed pigs. Corresponding recoveries for AGM-fed pigs were 10 and 90 g/100 g. These results suggest that SBTI have an inhibitory effect on the activity of pancreatic enzymes but do not cause an increase in their secretion.

Soybean trypsin inhibitors cause hypersecretion of pancreatic enzymes in rats and chicks but this effect has not been demonstrated in pigs (Li et al. 1997a). The high content of SBTI in soybean products such as Nutrisoy has a detrimental effect on enzyme activities by forming complexes with pancreatic enzymes which result in lower apparent ileal digestibilities of amino acids in pigs (Li et al. 1997b). If Nutrisoy is autoclaved, the content of SBTI is substantially reduced and apparent amino acid digestibilities are increased. The lower recoveries of endogenous amino acids in AGM-fed compared to UGM-fed pigs suggest that the SBTI formed complexes with pancreatic enzymes such as trypsin and chymotrypsin, thereby, increasing protein recoveries at the distal ileum. These results support the study by Li et al.(1997a) who showed that total activities of trypsin and chymotrypsin were not affected ($P > 0.05$) in pancreatic juice collected from pigs fed unprocessed compared to autoclaved Nutrisoy diets.

It is remarkable that other studies have not reported comparisons for amino acid content of guanidinated protein test meals and their respective diets. For this reason, it is uncertain if the differences in amino acid content between the test meals and diets are specific to Nutrisoy or to all protein sources. In this context, to ascertain whether the test meals were representative of the diets a comparison was made between the corresponding apparent amino acid digestibilities. Apparent ileal digestibilities of dry matter, crude protein and amino acids for the guanidinated test meals are presented in **Table 4**. The digestibility of crude protein was 33.7 percentage units higher ($P < 0.05$) for AGM compared to UGM. Corresponding amino acid digestibilities were also higher ($P < 0.05$) with differences ranging from 24.5 (aspartate + asparagine) to 61.3 (tyrosine) percentage units. With the exception of a lower ($P < 0.05$) methionine digestibility for UGM, the apparent digestibilities of all amino acids were similar ($P > 0.05$) between the guanidinated test meals and their respective diets. The apparent digestibilities of the

Table 4.

Apparent and true ileal digestibilities of amino acids in growing pigs fed unprocessed (UGM) and autoclaved (AGM) Nutrisoy guanidinated test meals.

	Apparent[1]			True[1]		
Item	UGM	AGM	SEM[2]	UGM	AGM	SEM[2]
			% of intake			
Dry matter	62.4	74.4	1.3	-	-	-
Crude protein	40.2	73.9	4.9	-	-	-
Amino acids						
Homoarginine	67.4	86.2	2.9	67.4	86.2	2.9
Indispensable						
Arginine	51.4	89.8	4.3	58.4	89.0	3.9
Histidine	53.8	83.2	4.9	69.3	85.2	2.7
Isoleucine	36.1	84.3	4.8	54.3	84.9	3.8
Leucine	38.4	86.6	4.6	48.3	86.6	4.1
Lysine[3]	52.6	73.4	5.0	59.2	77.6	3.5
Methionine[4]	41.5	74.3	3.6	59.6	78.7	2.4
Phenylalanine	41.5	88.9	4.2	56.2	88.3	3.7
Threonine	32.5	70.7	5.6	59.2	76.7	3.7
Valine	38.8	82.2	4.8	54.5	83.5	3.7
Dispensable						
Alanine	44.2	79.5	4.8	58.4	81.7	3.6
Aspartate + Asparagine	42.2	66.7	3.7	48.2	69.1	3.1
Cysteine	50.5	73.2	2.8	62.3	79.0	2.9
Glutamate + Glutamine	44.8	75.8	4.1	48.3	76.6	3.1
Glycine	34.3	69.9	5.1	54.1	75.7	4.1
Serine	34.5	76.6	4.3	53.4	79.4	4.0
Tyrosine	24.5	85.8	7.5	74.4	86.1	3.1

[1] Means in the same row, within apparent and true ileal digestibilities of the guanidinated test meals were always different ($P < 0.05$).

[2] Standard error of the mean (n = 6).

[3] Lysine digestibilities were calculated from the disappearance of residual lysine plus homoarginine. Apparent ileal digestibilities of only residual unguanidinated lysine for UGM and AGM were different ($P < 0.05$) from lysine in their respective diets at 24.8 and 65.0% (SEM; 3.3), respectively.

[4] The apparent ileal digestibility of methionine for the unprocessed Nutrisoy guanidinated test meal was different ($P < 0.05$) from the respective diet as reported by Li et al. (1997b).

respective diets were previously reported by Li et al. (1997b). The digestibilities of residual unguanidinated lysine in UGM and AGM were 24.8 and 65.0% (SEM: 3.3), respectively, and lower ($P < 0.05$) than corresponding digestibilities of lysine in the respective diets. This was expected as there would be a disproportionately greater contribution of endogenous to total lysine recovered at the distal ileum of pigs fed the guanidinated protein test meals compared to the respective diets. The similarity of apparent ileal amino acid digestibilities indicates that UGM and AGM were still representative of their respective diets even though there were some differences in amino acid content.

True ileal digestibilities of amino acids for the guanidinated test meals are presented in Table 4. True digestibilities were higher ($P < 0.05$) for AGM compared to UGM with differences ranging from 11.7 (tyrosine) to 38.3 (leucine) percentage units. True amino acid digestibilities for UGM were higher than the corresponding apparent digestibilities. These differences ranged from 3.5 (glutamate + glutamine) to 49.9 (tyrosine) percentage units. True amino acid digestibilities for AGM were also usually higher than their apparent values, although the differences were not as large and ranged from -0.6 (phenylalanine) to 6.0 (threonine) percentage units. The small difference between apparent and true amino acid digestibilities of AGM-fed compared to UGM-fed pigs suggest that SBTI mainly increased the recoveries of exogenous amino acids. However, true digestibilities of lysine were not different ($P > 0.05$) from the corresponding apparent digestibilities of homoarginine.

Trypsin preferentially hydrolyzes peptide linkages next to the basic amino acids. If homoarginine impedes the rate of proteolysis by trypsin then true amino acid digestibilities of the guanidinated test meals would be lower than that of the respective diets. Furthermore, endogenous amino acid recoveries determined as ratios to homoarginine are probably underestimated and therefore provide minimum estimates. Exogenous recoveries measured as the difference between total and endogenous flows of amino acids would be overestimated. This limitation of the ratio method is indicated by the negative values for endogenous recoveries of arginine and phenylalanine in AGM-fed pigs (Table 3). Although, the large differences in endogenous amino acid recoveries between unprocessed and autoclaved Nutrisoy suggests that the ratio method can be used to determine qualitative estimates. The effect of SBTI were clearly shown by large differences in endogenous amino acid recoveries between UGM- and AGM-fed pigs. These differences followed the pattern of appearance of amino acids from protein after enzymatic hydrolysis based on the specificities of the proteases and peptidases in the intestinal tract of the pig (Low, 1980). The lower content of SBTI in

AGM- compared to UGM-fed pigs decreased the endogenous recoveries of the basic, aromatic, branched-chain and hydroxy amino acids to a greater extent than of the other amino acids.

In general, amino acids that have a relatively higher content in intestinal and pancreatic secretions contributed a greater proportion to the sum of endogenous amino acids recovered from UGM-fed pigs. Pancreatic secretions have a high content of the branched-chain amino acids, glycine, aspartate and glutamate (Corring and Jung, 1972; Gabert et al., 1996). The same is true for serine and threonine which have a high content in intestinal mucins where they serve as attachment sites for oligosaccharide chains and for cysteine which is necessary to maintain conformation of the protein core (Dekker, 1990). In the AGM-fed pigs there was not a particularly high endogenous recovery of any amino acid. Presumably, digestion of protein of pancreatic and intestinal origin was not inhibited in the small intestine of the AGM-fed pigs to the same extent as the UGM-fed pigs because of the lower content of SBTI in AGM.

In conclusion, guanidination of Nutrisoy changed the amino acid composition of the test meals with respect to the diets. The homoarginine ratio method was an effective approach which provided qualitative differences of endogenous and exogenous recoveries of amino acids from the distal ileum of pigs fed Nutrisoy diets differing in the content of SBTI. Further studies to find methods which provide quantitative estimates are warranted.

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Literature Cited

AOAC. 1990. Official Methods of Analysis. (14th Ed.). Association of Official Analytical Chemists. Washington, DC.

Barth, C. A., Lunding, B., Schmitz, M. & Hagemeister, H. (1993) Soybean trypsin inhibitor(s) reduce absorption of exogenous and increase loss of endogenous protein in miniature pigs. J. Nutr. 123: 2195-2200.

Butts, C. A., Moughan, P.J., Smith, W.C. & Carr, D.H. (1993) Endogenous lysine and other amino acid flows at the terminal ileum of the growing pig (20 kg bodyweight): the effect of protein-free, synthetic amino acid, peptide and protein alimentation. J. Sci. Food Agric. 61: 31-40.

CCAC. 1980. Canadian Council on Animal Care. Guide to the Care and Use of Experimental Animals. Vol 1 (with addendum). Ottawa, Ontario.

Chung, T.K. & Baker, D.H. (1992) Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. J. Anim. Sci. 70: 3781-3790.

Corring, T. & Jung., J. (1972) The amino acid composition of pig pancreatic juice. Nutr. Rep. Int. 6: 187-190.

Dekker, J. (1990) Gastric mucin, structure and biosynthesis. PhD. Thesis, Rijksuniversiteit Utrecht, Utrecht, The Netherlands.

Gabert, V. M., Sauer, W.C., Li, S. & Fan, M.Z. (1996) Exocrine pancreatic secretions in young pigs fed diets containing fababeans (*Vicia faba*) and peas (*Pisum sativum*): concentrations and flows of total, protein-bound and free amino acids. J. Sci. Food Agric. 70: 256-262.

Furuya, S. & Kaji, Y. (1986) True ileal digestibility of crude protein and amino acids in protein sources as determined by a regression method for growing pigs. Jpn. J. Zootech. Sci. 57: 859-870.

Hagemeister, H. & Erbersdobler, H.F. (1985) Chemical labelling of dietary protein by transformation of lysine to homoarginine: a new technique to follow intestinal digestion and absorption. Proc. Nutr. Soc. 44: 133A.

Imbeah, M., Sauer, W.C. & Caine, W.R. (1995) Comparison of the single dose and withdrawal method for measuring the rate of passage of two digestibility markers in digesta collected from the distal ileum and feces in growing pigs. Anim. Feed Sci. Technol. 52: 41-50.

Imbeah, M., Angkanaporn, K., Ravindran, V. & Bryden, W.L. (1996) Investigation on the guanidination of lysine in proteins. J. Sci. Food Agric. 72: 213-218.

Jones, B. N. & Gilligan, J.P. (1983) O-Phthaldialdehyde precolumn derivitization and reverse-phase high performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J. Chromatogr. 266: 471-482.

Kennelly, J. J., Aherne, F.X. & Apps, M.J. (1980) Dysprosium as an inert marker for swine digestibility studies. Can. J. Anim. Sci. 60: 441-446.

de Lange, C. F. M., Sauer, W.C. & Souffrant, W.B. (1989) The effect of 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. 67: 755-762.

de Lange, C.F.M., Sauer, W.C., Souffrant, W.B. & Lien, K.A. (1992) ^{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. J. Anim. Sci. 70: 1848-1856.

Li, S., Sauer, W.C., Huang, S. & Hardin, R.T. (1997a) Response of pancreatic secretions to feeding diets with low and high levels of soybean trypsin inhibitors in growing pigs. J. Sci. Food Agric. (in press).

Li, S., Sauer, W.C. & Caine, W.R. (1997b) Response of nutrient digestibilities to feeding diets of low and high levels of soybean trypsin inhibitors in growing pigs. J. Sci. Food Agric. (in press).

Low, A. G. (1980) Nutrient absorption in pigs. J. Sci. Food Agric. 31: 1087-1130.

Maga, J.A. (1981) Measurement of available lysine using the guanidination reaction. J. Food Sci. 46: 132-134.

Marty, B. J., Chavez, E.R. & de Lange, C.F.M. (1994) Recovery of amino acids at the distal ileum for determining apparent and true ileal amino acid digestibilities in growing pigs fed various heat-processed full-fat soybean products. J. Anim. Sci. 72: 2029-2037.

Moughan, P. J., & Rutherfurd, S.M. (1990) Endogenous flow of total lysine and other amino acids at the terminal ileum of protein- or peptide-fed rats: the chemical labelling of gelatin protein by transformation of lysine to homoarginine. J. Sci. Food Agric. 52: 179-192.

National Research Council. 1988. Nutrient Requirements of Swine. (9th rev. Ed.) National Academy of Sciences-National Research Council, Washington, D.C.

Petersen, R.G. 1985. Design and Analysis of Experiments. pp. 305-306. Marcel Dekker, Inc. New York and Basel.

Rutherfurd, S. M. & Moughan, P.J. (1990) Guanidination of lysine in selected dietary proteins. J. Agric. Food Chem. 38: 209-211.

SAS. 1990. SAS/STAT® User's Guide (Release 6.08). SAS Inst. Inc., Cary, NC.

Schmitz, M., Hagemeister, H. & Erbersdobler, H.F. (1991) Homoarginine labelling is suitable for determination of protein absorption in miniature pigs. J. Nutr. 121: 1575-1580.

Schulze, H., Saini, H.P., Huisman, J., Hessing, M., van den Berg, W. & Verstegen, M.W.A. (1995) Increased nitrogen secretion by inclusion of soya lectin in the diets of pigs. J. Sci. Food. Agric. 69: 501-510.

Siriwan, P., Bryden, W.L. & Annison, E.F. (1994) Use of guanidinated dietary protein to measure losses of endogenous amino acids in poultry. Br. J. Nutr. 71: 515-529.

Steel, R. G. D. & Torrie, J.H. (1980) Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Publishing Co., New York.

2 Effect of Protease Treatment of Soybean Meal On Content of Total Soluble Matter and Crude Protein and Level of Soybean Trypsin Inhibitors

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Abstract

An *in vitro* study was undertaken to determine the effect of treating soybean meal with protease at different temperature and pH conditions on the content of total soluble matter and crude protein (CP) and the level of soybean trypsin inhibitors (SBTI). Soybean meal (5 g), weighed into 25 × 150 mm culture tubes, was suspended in 20 mL of distilled water, adjusted to pH 3, 4.5 or 6, with *Bacillus subtilis* subtilisin-protease (Finnfeeds International Ltd.) at 0, 0.2 or 1.0 mg g^{-1} soybean meal. The culture tubes were incubated for 16 h in a temperature-controlled water bath at 40 or 50°C. The soluble and insoluble fractions of soybean meal were separated by centrifuging the culture tubes and decanting the supernatant into separate conical tubes. All tubes were freeze-dried and the contents analyzed for CP. The content of total soluble matter and CP, on a dry matter basis, were determined as the difference between the beginning and end weight of the culture tubes. The content of total soluble matter and CP and the level of SBTI were usually similar ($P > 0.05$) for soybean meal treated with protease at 0 or 0.2 mg g^{-1}. Protease pretreatment at 1.0 mg g^{-1} soybean meal increased ($P < 0.05$) the content of soluble CP and, with the exception of incubation at 50°C and pH 6, lowered ($P < 0.05$) the level of SBTI compared with soybean meal pretreated with protease at 0 or 0.2 mg g^{-1} under all conditions of temperature and pH. Based on the results of this *in vitro* trial, protease pretreatment at 1.0 mg g^{-1} soybean meal has the potential to improve the availability and digestion of soybean meal protein in diets for pigs.

Key words: Soybean Meal, Protease, Soybean Trypsin Inhibitors

Introduction

Raw soybeans have a high content of antinutritional factors (ANF), especially soybean trypsin inhibitors (SBTI), which are partially inactivated during the solvent-extraction and toasting process (Marty, 1994). The remaining soybean meal is a high quality source of protein. Pretreatment of soybean meal with protease may increase the proportion of soluble crude protein (CP), thereby, potentially increasing protein digestibility in young pigs. However, the utilization of nutrients and energy by young pigs fed diets containing soybean meal may be affected by the residual ANF (Tamminga et al., 1995). Schulze (1994) reported increasing flows of nitrogen (N) at the terminal ileum of 13 kg pigs fed a cornstarch-based soya concentrate diet with increasing levels of purified SBTI. The residual SBTI in soybean meal are proteins and, therefore, likely susceptible to degradation by proteases. Other ANF which contain protein such as antigenic soybean proteins (glycinin and β-conglycinin) or soy lectins are also likely to be partly inactivated by protease treatment. The effects of *in vitro* incubation of soybean meal with protease under different conditions of temperature and pH on the content of total soluble matter and CP and the level of SBTI were evaluated.

Experimental Procedures

All *in vitro* incubation conditions were performed in quadruplicate using 25 × 150 mm culture tubes. Eighteen different incubation conditions of temperature, pH and *Bacillus subtilis* subtilisin-protease (Finnfeeds International Ltd.) were evaluated. Conditions were, as follows: water bath temperatures of 40 and 50°C; incubation solutions with pH 3, 4.5 and 6 and protease concentrations of 0, 0.2 and 1.0 mg g^{-1} soybean meal. Five g of dehulled solvent-extracted and toasted soybean meal (537.2 g CP kg^{-1}, dry matter basis), obtained from a commercial supplier, were weighed into the culture tubes and 20 mL of previously prepared incubation solutions added. The incubation solutions were prepared using deionized water adjusted to pH using 6N HCl to which protease was added. The contents were thoroughly mixed before placing the culture tubes in a temperature-controlled water bath for 16 h. At least once during the incubation period the tubes were mixed again.

At the conclusion of the incubation period, the contents in the culture tubes were mixed, then centrifuged at 4,500 × *g* for 10 min. The supernatant from the culture tubes was decanted into 50 mL conical tubes. Soluble matter still remaining in the culture tubes was further extracted by addition of 12 mL of distilled water, mixed and then centrifugated at 4,500 × *g* for 10 min. The supernatant from the second extraction was decanted into the same conical tubes. The conical tubes were sealed with parafilm and then frozen at -20°C. The culture tubes containing the remaining soybean meal were also frozen at -20°C. The contents of both sets of tubes were freeze-dried. Following freeze-drying, the amount of soybean meal remaining in the culture tubes (dry matter basis; DM) was considered to be the total amount of insoluble matter. Part of the contents of some of the conical tubes were lost due to foaming during freeze-drying. Therefore, difference in the weight of DM in the culture tubes before and after the incubation periods and extraction was assumed to be the total amount of soluble matter.

Prior to subsequent analyses, the insoluble and soluble matter in the culture and conical tubes, respectively, were finely ground using a mortar and pestle. Analysis for DM was carried-out according to the Association of Official Analytical Chemists (1990). Nitrogen analysis was performed using an Automatic Nitrogen Analyzer (FP-428 Nitrogen Determinator, Leco® Corporation, St Joseph, MI). The DM and CP (nitrogen × 6.25) analyses were carried-out in duplicate on the contents of all of the culture and conical tubes. Level of SBTI were determined in triplicate on pooled samples (within the different incubation conditions) of the soybean meal remaining in the culture tubes according to the procedure described by Hamerstrand et al. (1981).

The data were subjected to analysis of variance using the General Linear Model procedure of the Statistical Analysis Systems Institute (SAS, 1990). The statistical model included protease, temperature and pH as main effects and interactions tested against the error term. Means for protease treatments within the different conditions of temperature and pH, were separated with the Student Newman-Keuls multiple range test (Steel and Torrie, 1980). The means for the content of total soluble matter and CP and the level of SBTI were based on four and three observations, respectively. The content of total soluble matter and CP and the level of SBTI for untreated solvent-extracted and toasted soybean meal (not subjected to any incubation conditions) were compared to results from samples subjected to the various incubation conditions using a dependent (paired) two-tailed t-test (Steel and Torrie, 1980).

Results and Discussion

The content (DM basis) of total soluble matter and CP and the level of SBTI in soybean meal treated at different protease concentrations, temperatures and pH are presented in **Table 1**. These values represent maximum estimates as they do not take into account the proportion of soybean meal lost as volatile products.

Solubility of total matter

With the exception of the treatment at 40°C and pH 6, the content of total soluble matter from soybean meal treated with protease at 1.0 mg g^{-1} was increased ($P < 0.05$) under all conditions of temperature and pH compared to 0 or 0.2 mg g^{-1} protease treatment. The highest content of total soluble matter was 356.0 g kg^{-1} for soybean meal treated with protease at 1.0 mg g^{-1} at 50°C and pH 4.5. The increases ($P < 0.05$) in total soluble matter for soybean meal treated with protease at 1.0 versus 0 mg g^{-1} ranged from 62.0 g kg^{-1} at 40°C and pH 3 to 143.6 g kg^{-1} at 50°C and pH 4.5. Protease treatment at 0.2 versus 0 mg g^{-1} soybean meal did not improve ($P > 0.05$) the content of total soluble matter under any temperature and pH conditions. The total soluble matter of untreated soybean meal, extracted in deionized water (pH 7), was 184.3 g kg^{-1} (SE: 4.5; n = 4) and usually similar ($P > 0.05$) to soybean meal treated with protease at 0 or 0.2 mg g^{-1}.

Table 1
Effect of different *in vitro* incubation conditions of protease concentration, temperature and pH on the content[a] of total soluble matter and crude protein (CP) and the level[a] of soybean trypsin inhibitors (SBTI) in soybean meal

	Protease (mg g^{-1} soybean meal)			
Incubation conditions	0	0.2	1.0	SEM[b]
Soluble matter (g kg^{-1}):				
Temperature 40°C				
pH 3	192.4^{d}	203.8^{d}	254.4^{c}	7.5
pH 4.5	200.4^{d}	201.2^{d}	268.1^{c}	10.1
pH 6	202.3	233.9	254.0	16.1
Temperature 50°C				
pH 3	207.6^{d}	224.9^{d}	338.9^{c}	10.3
pH 4.5	212.4^{d}	228.9^{d}	356.0^{c}	6.0
pH 6	218.7^{d}	239.1^{d}	338.8^{c}	7.1
Soluble CP (g kg^{-1}):				
Temperature 40°C				
pH 3	98.0^{d}	124.3^{d}	183.0^{c}	14.1
pH 4.5	100.5^{d}	93.0^{d}	185.5^{c}	11.2
pH 6	96.0^{d}	113.5^{d}	171.0^{c}	8.0
Temperature 50°C				
pH 3	82.5^{d}	107.7^{d}	311.0^{c}	9.6
pH 4.5	90.5^{e}	113.0^{d}	318.7^{c}	3.4
pH 6	84.7^{d}	116.0^{d}	297.3^{c}	14.5
SBTI (mg kg^{-1}):				
Temperature 40°C				
pH 3	4.38^{c}	3.21^{d}	2.51^{e}	0.15
pH 4.5	3.20^{c}	3.29^{c}	2.79^{d}	0.09
pH 6	3.21^{d}	3.63^{c}	2.48^{e}	0.09
Temperature 50°C				
pH 3	3.65^{c}	3.53^{c}	2.16^{d}	0.17
pH 4.5	3.55^{c}	3.17^{d}	3.08^{d}	0.11
pH 6	3.43	3.88	3.70	0.14

[a] Dry matter basis.
[b] Standard error of the mean (n = 4; for total soluble matter and CP, whereas n = 3 for level of SBTI).
[c,d,e] Values in the same row with different superscripts differ ($P < 0.05$).

Solubility of crude protein

The protease used in this experiment contained 65 g kg^{-1} of CP. As such, differences in protease addition to the incubation solutions did not contribute an appreciable amount to differences in CP among the treatments. The content of soluble CP in soybean meal treated with protease at 1.0 mg g^{-1} was increased ($P < 0.05$) under all temperature and pH conditions compared to 0 or 0.2 mg g^{-1}. The increases in soluble CP in soybean meal treated with protease at 1.0 versus 0 mg g^{-1} ranged from 75.0 g kg^{-1} for soybean meal treated at 40°C and pH 6 to 228.2 g kg^{-1} at 50°C and pH 4.5. Incubation with protease at 0.2 versus 0 mg g^{-1} soybean meal at 50°C and pH 4.5 also increased ($P < 0.05$) the content of soluble CP. The content of soluble CP of untreated soybean meal, extracted in deionized water (pH 7), was 97.5 g kg^{-1} (SE: 2.7; n = 4) and usually similar ($P > 0.05$) to values for soybean meal treated with protease at 0 or 0.2 mg g^{-1}.

Level of soybean trypsin inhibitors

With the exception of incubation at 50°C and pH 6, the level of SBTI decreased ($P < 0.05$) in soybean meal treated with protease at 1.0 compared to 0 mg g^{-1} under all temperature and pH conditions. At 50°C and pH 4.5 protease concentrations at both 0.2 and 1.0 mg g^{-1} soybean meal reduced ($P < 0.05$) the level of SBTI by about 13% compared to soybean meal without protease treatment (0 mg g^{-1}). The level of SBTI in untreated soybean meal was 3.66 mg kg^{-1} (SE: 0.15) and, with the one exception, was similar ($P > 0.05$) to soybean meal treated with protease at 0 or 0.2 mg g^{-1} under all temperature and pH conditions. On the other hand, with the exception of incubation at 50°C and pH 6, the level of SBTI in soybean meal treated with protease at 1.0 mg g^{-1} was lower ($P < 0.05$) than untreated soybean meal under all temperature and pH conditions. Based on the similar ($P > 0.05$) level of SBTI in soybean meal treated with protease at 0 or 0.2 mg g^{-1} and untreated, it was assumed that the soluble matter did not contain active SBTI. However, peptide fragments and free amino acids from proteolysis of the SBTI may have been contained in the soluble fraction of soybean meal treated with protease at 1.0 mg g^{-1}.

There is a scarcity of information on the effect of protease treatment of soybean meal on the content of other ANF which have a protein component. For instance, lectins which are glycoproteins and bind to the terminal N-acetyl-D-galactosamine and -D-galactose of the intestinal glycocalyx and mucins reduce the digestion and absorption of nutrients (Schulze, 1994). The addition of purified soya lectins at a level of 960 mg kg^{-1} to a cornstarch-based soya concentrate diet increased total nitrogen flow at the

terminal ileum of 13 kg pigs by 360 mg kg^{-1} of DM intake (Schulze et al., 1995). The presence of immunologically-active antigenic soybean proteins such as glycinin and β-conglycinin in soybean meal may cause digestive disorders and diarrhea due to transient gut hypersensitivity (higher anti-soya immunoglobulin G titers) and decrease in villus height in duodenal tissue of newly weaned piglets (Li et al., 1991). As a result, the piglets have poor growth performance in the first weeks after weaning. In this context, Rooke et al. (1996) reported a 15% decrease in antigenic soy-proteins in corn soybean meal-based diets in which the soybean meal was pretreated with protease (1 g kg^{-1} DM) at pH 4.5. Average daily gain was increased ($P < 0.05$) during the first 7 d post-weaning period in three week old piglets fed the corn protease-treated soybean meal diet.

The SBTI are the primary ANF in soybeans (Schulze, 1994). As such, apparent digestibilities of DM and nitrogen were reported to increase in pigs fed raw soybeans

Table 2
Effect of different concentrations of protease under all conditions of temperature and pH on the content[a] of total soluble matter and crude protein (CP) and the level[a] of soybean trypsin inhibitors (SBTI) in soybean meal

	Soluble matter (g kg^{-1})	Soluble CP (g kg^{-1})	SBTI (mg kg^{-1})
Protease (mg g^{-1})			
0	205.6	92.0	3.57
0.2	222.0	111.2	3.45
1.0	301.7	244.4	2.79
SEM[b]	7.2	4.5	0.08
Significance level of:			
Protease (P)	0.0001	0.0001	0.0001
pH	0.25	0.46	0.02
Temperature (T)	0.0001	0.0001	0.01
P × pH	0.18	0.19	0.0001
P × T	0.0001	0.0001	0.02
pH × T	0.83	0.51	0.0001

[a] Dry matter basis.
[b] Standard error of the mean (n = 24 for total soluble matter and CP; n = 18 for level of SBTI).

when the levels of SBTI were decreased by thermal processing (Qin et al., 1996). Marty (1994) reported that the solvent-extraction and toasting process reduced the SBTI level from about 180 mg kg^{-1} in full-fat soybeans to 4 mg kg^{-1} in soybean meal. In the present study, soybean meal treated with protease at 0.2 mg g^{-1} had a SBTI content only slightly lower than the 4 mg kg^{-1} soybean meal reported by Marty (1994). Whereas, protease at 1.0 mg g^{-1} soybean meal reduced the level of SBTI by about 25% compared to untreated soybean meal. In this context, protease pretreatment at 1.0 mg g^{-1} compared to untreated soybean meal may have the potential to improve the availability and digestion of protein in young pigs. This is in agreement with the improved growth performance of newly weaned piglets fed soybean meal pretreated with protease (Rooke et al., 1996).

The overall effect of protease concentration on the content of total soluble matter and CP and level of SBTI in soybean meal are summarized in **Table 2**. Protease concentration (1.0 mg g^{-1} soybean meal) was the most important factor for increasing the content of total soluble matter and CP and lowering the level of SBTI in soybean meal. Temperature and pH conditions were less important.

In conclusion, under the conditions of this experiment protease pretreatment at 1.0 mg g^{-1} soybean meal at 50°C and pH 4.5 would appear to have the best potential for improving the availability and digestion of protein and lowering the level of SBTI in diets for pigs.

Acknowledgments

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Literature Cited

Association of Official Analytical Chemists 1990. Official Methods of Analysis. (14th Ed.). Association of Official Analytical Chemists. Washington, DC.

Hamerstrand, G.E., Black, L.T. and Glover, J.D., 1981. Trypsin inhibitors in soy products: Modification of the standard analytical procedure. Cereal Chemistry, 58: 42-45.

Li, D.F., Nelssen, J.L., Reddy, P.G., Blecha, F., Klemm, R. and Goodband, R.D., 1991. Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci., 69: 4062-4069.

Marty, J.M., 1994. Nutritional evaluation of various processed soybean products with growing pigs and rats. PhD. Thesis, McGill University, Montreal, Quebec, Canada.

Qin, G., ter Elst, E.R., Bosch, M.W. and van der Poel, A.F.B., 1996. Thermal processing of whole soya beans: Studies on the inactivation of antinutritional factors and effects on ileal digestibility in piglets. Anim. Feed Sci. Technol., 57: 313-324.

Rooke, J.A., Fraser, H., Shanks, M. and Morgan, A., 1996. The potential for improving soya-bean meal in diets for weaned piglets by protease treatment: comparison with other protein sources. Proceedings of the 112th meeting of the British Society of Animal Science, Scarborough, UK. Anim. Sci., 62: 658.

Schulze, H., 1994. Endogenous ileal nitrogen losses in pigs: dietary factors. PhD. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Schulze, H., Saini, H.S., Huisman, J., Hessing, M., van den Berg, W. and Verstegen, M.W.A., 1995. Increased nitrogen secretion by inclusion of soya lectin in the diets for pigs. J. Sci. Food Agric., 69: 501-510.

SAS 1990. Statistical Analysis Systems. SAS/STAT User's Guide (Release 6.08). Statistical Analysis Systems Institute Inc., Cary, NC.

Steel, R.G.D. and Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Book Publishing Co., New York.

Tamminga, S., Schulze, H., van Bruchem, J. and Huisman, J., 1995. The nutritional significance of endogenous N-losses along the gastro-intestinal tract of farm animals. Arch. Anim. Nutr., 48: 9-22.

3 Apparent Ileal Digestibilities of Amino Acids in Newly Weaned Piglets Fed Diets With Protease-Treated Soybean Meal

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Abstract

Apparent ileal digestibilities of amino acids were determined in piglets fed cornstarch-based diets with untreated or protease-treated soybean meal as protein source. Sixteen piglets were fitted with a modified post valve T-cecum cannula on d 14, 15 and 16 after birth and then returned to their sows until d 20 when they were weaned. Twelve of the piglets were selected for the study, which was conducted according to a two period balanced change-over design. Treatments consisted of soybean meal which was untreated (SBM), processed by incubation (1:2, wt/vol distilled water adjusted to pH 4.5, for 16 h at 50°C; CI-SBM), sprayed with protease (supplied at 1 µL/g of soybean meal; PS-SBM) and processed by incubation, as previously described, with protease (supplied at 1 µL/g of soybean meal; PI-SBM). Each period consisted of 5 d adaptation to diets followed by three 8-h collection periods (total of 24 h) that alternated with 8-h periods in which digesta was not collected. Apparent CP digestibilities (%) were similar ($P > .05$) at 70.4, 72.4, 65.2 and 70.3 for the SBM, CI-SBM, PS-SBM and PI-SBM diets, respectively. Corresponding amino acid digestibilities were also similar ($P > .05$), ranging from 62.5, 67.5, 57.9 and 65.0 for alanine to 83.5, 83.4, 78.7 and 84.7 for arginine. Apparent digestibilities were lower ($P < .05$) for Period 1 (on d 7 after weaning) compared with Period 2 (on d 16 after weaning). In conclusion, protease treatment of soybean meal had no effect on ileal digestibilities of CP and amino acids in newly weaned piglets.

Key Words: Piglets, Amino Acids, Protease, Ileal Digestibilities

Introduction

Overcoming growth depression in newly weaned piglets the first few weeks after weaning is an important consideration for improving swine production. The ability of newly-weaned piglets to assimilate nutrients from non-milk based diets is compromised by a delay in the adaptation of the gastrointestinal tract to secrete sufficient enzymes to fully support digestive processes (Corring, 1980; Makkink and Verstegen, 1990). Morphological studies have also shown that transient hypersensitivity and atrophy of the intestinal mucosa at weaning greatly reduces the capacity of piglets to digest plant-based protein sources such as soybean meal (Li et al., 1990, 1991a, b; Kik, 1991).

Several companies are developing enzymes which are specific for use in the swine feed industry (Partridge and Schulze, 1996). Little is known about the efficacy of proteases in these enzyme preparations on dietary protein utilization. The *in vitro* incubation of soybean meal with protease increases CP solubility and reduces the content of soybean trypsin inhibitors (**SBTI**; Caine et al., 1997). The presence of SBTI causes inactivation and hypersecretion of pancreatic proteolytic enzymes and subsequent loss of endogenous protein in monogastric animals (Tamminga et al.,

1995). Therefore, treatment of soybean meal with protease might improve CP and amino acid digestibilities in newly weaned piglets.

The objective of the present study was to determine the effect of protease treatment of soybean meal, administered either as a topical spray or via incubation, on the apparent ileal digestibilities of CP and amino acids in piglets in the 18 d after weaning at 20 d of age.

Experimental

Animals and Management

Pre-weaning management of piglets. At birth, ten male and six female piglets with an average birth weight of 1.8 ± .1 kg were cross-fostered onto two second parity PIC sows (Camborough × Canabrid) which were housed in farrowing pens at the University of Alberta Swine Research Centre. Eight piglets were matched onto each sow to make-up uniform groups to minimize suckling and sibling rivalry. Thereafter, the piglets were handled by normal management practices. On the day after birth, the pigs were weighed, they were injected with iron and oxytetracycline, and their tails were docked. On d 5 after birth the male piglets were castrated. During d 14, 15 and 16 after birth, piglets were taken from the sows and prevented from nursing for at least 3 h prior to surgery and fitted with a modified post valve T-cecum cannula (**mPVTC**). Of the eight piglets from each sow, only three underwent surgery on a given day. The average BW of the piglets prior to surgery was 5.3 ± .2 kg.

Placement of the mPVTC cannula in piglets. A modified version of the post valve T-cecum cannula described by van Leeuwen et al. (1991) was developed for use in piglets. The cannula was made from Plastisol (Techniplast, F.H. and Sons Mfg. Ltd., Mississauga, ON) and consisted of a single flange and barrel set at an angle of fifteen degrees. The flange had a diameter of 35 mm and a thickness of 5 mm. The barrel was 45 mm in length with an i.d. of 15 mm and o.d. of 18 mm. A solid plug, 14 mm in diameter, running the full length of the barrel was made from Plastisol. Once fitted to the piglet the cannula was held in place against the body wall with a slightly larger cover flange.

Surgical placement of the mPVTC was similar to that described by van Leeuwen et al. (1991) with two modifications. Firstly, the apex region was not transsected because of the small size of the cecum in young piglets. Instead, the cannula was positioned through the serosal layer of the lower segment of the corpus region of the cecum leaving approximately 2 to 3 cm of the apex tucked-up on top of the flange

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

	Control diets		Protease-treated diets	
	SBM	CI-SBM	PS-SBM	PI-SBM
Corn starch	29.1	29.7	28.1	30.3
Soybean meal[c]	43.2	42.6	44.2	42.0
Dextrose	10.0	10.0	10.0	10.0
Cellulose[d]	6.0	6.0	6.0	6.0
Canola oil	5.0	5.0	5.0	5.0
Biophos[e]	3.5	3.5	3.5	3.5
Vitamin-mineral premix[f]	2.0	2.0	2.0	2.0
DL-Methionine	.4	.4	.4	.4
Fortified salt[g]	.3	.3	.3	.3
Chromic oxide	.3	.3	.3	.3
Antibiotic[h]	.2	.2	.2	.2

[a] As-fed basis.

[b] Soybean meal which was untreated (SBM) and incubated (1:2, wt/vol in distilled water at 50°C and pH 4.5; CI-SBM) were the two "Control diets" or sprayed (PS-SBM) and incubated (PI-SBM) with protease (1 µL/g of soybean meal) were the two "Protease-treated diets".

[c] Contained the following (g/kg): DM, 902.0; CP, 479.5; Ash, 59.9. The soybean trypsin inhibitors content was 3.66 mg/kg of soybean meal. Supplied by New-Life Feeds, Parrish and Helmbecker Ltd., Edmonton, AB.

[d] Solka-Floc; powdered cellulose. Supplied by James River Corp., Berlin, NH.

[e] Provided (g/kg): available phosphorus, 150 to 180; calcium, 240. Supplied by Continental Lime Ltd., Exshaw, AB.

[f] Provided the following (mg/kg of diet): retinyl palmitate, 4.54; cholecalciferol, .025; α-tocopheryl acetate, 80.0; menadione, 4.0; cyanocobalamin, .06; riboflavin, 24.0; niacin, 80.0; dl-pantothenate, 50.0; choline, 1200.0; d-biotin, .5; folacin, 3.2; thiamin, 6.0; ethoxyquin, 10.0; Fe, 300.0; Mn, 40.0; Zn, 240.0; Cu, 250.0; I, .4; Se, .3. Supplied by Hoffmann-LaRoche Ltd., Meadowpine Bvd., Mississauga, ON.

[g] Provided (g/kg): NaCl, 950; Na, 380; I, .15; Co, .05; Cu, 3.5; Mn, 10.0; Zn, 9.0; Se, .075. Supplied by Champion Feed Services Ltd., Westlock, AB.

[h] Veterinary LS-20 premix. Provided the following (g/kg of premix): Lincomycin hydrochloride, 22; Spectinomycin sulphate, 22; mineral oil USP, 10; soybean meal carrier, 946. Supplied by The Upjohn Company, Animal Health Division, Orangeville, ON.

around the barrel. Secondly, the barrel was exteriorized through a fistula in the intercostal region between the last two ribs. Proper positioning of the cannula was verified by observation of the ileo-cecal valve through the barrel.

Post-surgical care of the piglets. Immediately after surgery the piglets were returned to the farrowing pens and continuously monitored until they had recovered sufficiently to rejoin their littermates. This approach was intended to cause minimum stress to the sow and to prevent a significant reduction in milk production. Piglets were usually suckling within 8 h after surgery. On d 17 a cereal-based creep feed (wheat: 27%; oat groats: 20%; soybean meal: 16%; whey powder: 15%; barley: 10%; fish meal: 4%; tallow: 4% and the remaining 4% was minerals, vitamin-mineral premix and antibiotic premix) was made available to the piglets until weaning. The piglets were weaned at 20 d of age, transferred to the Swine Metabolic Research Facility and placed into individual metabolism crates (height; 85 cm, length; 70 cm and width; 65 cm) located in a room in which the temperature was maintained between 28 to 32°C. Water was freely available from a low-pressure drinking nipple. The average BW of piglets at weaning was 6.2 ± .3 kg.

Preparation of the diets. The composition of the experimental diets is presented in **Table 1**. The experimental diets were cornstarch-based with untreated or protease-treated (*Bacillus subtilis* subtilisin-protease; Finnfeeds International Ltd.) soybean meal as protein source. Four different forms of soybean meal (48% CP) from a single commercial source were prepared. Soybean meal which was untreated (**SBM**) or incubated (1:2, wt/vol in distilled water with pH adjusted to 4.5 with HCl and then heated for 16 h at 50°C; **CI-SBM**) were the two "Control diets". Soybean meal sprayed at 50 mL/kg with distilled water (adjusted to pH 4.5) which contained the protease supplied at 1 µL/g of soybean meal (**PS-SBM**) or incubated the same as described for the CI-SBM treatment, except that the water solution supplied protease at 1 µL/g of soybean meal (**PI-SBM**) were the two "Protease-treated diets". The incubation conditions used in the present study were based on *in vitro* increases ($P < .05$) in CP solubility and decrease ($P < .05$) in SBTI content of SBM, previously reported by Caine et al. (1997).

Soybean meal for the PS-SBM treatment was prepared by mixing 5 kg batches while spraying with a protease solution and then allowing the meal to air dry for 24 h. The incubation treatments were prepared by adding 8 L of distilled water buffered to a pH of 4.5 with HCl, with or without protease, into plastic pails and then slowly adding and stirring in 4 kg of soybean meal until a mash was formed. The pails were sealed to prevent loss of volatile products and then placed in a forced-air draft oven set at 50°C

for 16 h. At the conclusion of the incubation process the contents of the pails were immediately transferred into stainless steel trays, frozen at -20°C and then freeze-dried. The resultant cakes were subsequently crushed by hand through a sieve with a 2.36-mm mesh.

Diets were formulated to contain 20% CP. Canola oil was added so that the diets exceeded NRC (1988) standards for digestible energy. Vitamins, minerals and DL-methionine were also included in the diets to meet or exceed NRC (1988) standards. Chromic oxide (.3%) was included as a digestibility marker.

Experimental Protocol. The experiment was carried out according to a two-period changeover design for four treatments (Gill and Magee, 1976). Each experimental period lasted 9 d. Piglets were weaned in the late evening at 20 d of age, placed in metabolism crates and offered 50 g of the creep feed overnight. In the morning, twelve of the most vigorous piglets were randomized, according to weight, sex and sow, to one of the four dietary treatments. Feed was offered to the piglets at 8-h intervals at 0800, 1600 and 2400 h. Each piglet was offered fresh feed at each meal. Remaining feed was removed and weighed just before the subsequent meal. Ileal digesta were collected for a total of 24 h in alternating 8-h periods: from 0800 to 1600 h on d 6 and 2400 to 0800 h and 1600 to 2400 h on d 7. The contents in the cecum were collected for a period of 45 min prior to the start of each collection period and discarded. Digesta were collected into soft plastic bags (length: 10 cm; i.d. 1.5 cm) attached to the barrel of the cannula. A 5 mL solution of formic acid (10%, vol/vol) was added to each bag to minimize bacterial activity. Bags were changed at least every hour and the contents pooled within piglet and period and immediately frozen at -20°C until analyses. The experimental period was continued for an additional 3 d in conjunction with another study to determine the endogenous recoveries and true ileal digestibilities of amino acids using guanidinated protein. At the conclusion of the first experimental period the dietary treatments for the piglets were switched according to the experimental design and the second period repeated as previously described.

The experimental proposal and surgical procedures were reviewed and approved by the Animal Care Committee of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta and the Wageningen Institute of Animal Science at the Wageningen Agricultural University. The animals were cared for in accordance with the guidelines established by CCAC (1980).

Analytical and Statistical Procedures

Chemical analyses. Samples of digesta were freeze-dried and ground through a .5-mm mesh screen in a Wiley mill (Arther H. Thomas Co., Philadelphia, PA) before analyses. Diets were ground similarly. The DM content of samples were determined according to AOAC (1990). The energy content of the diets were determined using a Parr 1241 Adiabatic Bomb Calorimeter (Parr Instrument Co., Moline, IL). The N contents of the diets and digesta were measured with an Automated N Analyzer (FP-428 Nitrogen Determinator, Leco® Corporation, St. Joseph, MI). The chromic oxide content was determined using the procedure described by Fenton and Fenton (1979).

Contents of amino acids in diets and digesta were analyzed as o-phthaldialdehyde derivatives by HPLC according to the procedure of Jones and Gilligan (1983) using a Varian 5000 HPLC system with a Varian 3300 autosampler and a Varian Fluorichrom detector (excitation 340 nm, emission 450 nm; Varian Canada Inc., Mississauga, ON). Samples were run on a Supelcosil 3 micron LC-18 reverse phase column (4.6 × 150 mm; Supelco, Sigma-Aldrich Canada Ltd., Mississauga, ON) equipped with a Supelco LC-18 reverse phase 20 to 40 micron guard column (4.6 × 50 mm). The diet and digesta samples were hydrolyzed for 24 h with 6N HCl prior to HPLC procedures. Methionine and cysteine were determined as methionine sulphone and cysteic acid, respectively, after oxidation with performic acid according to AOAC (1990). The oxidized samples were dried and then hydrolyzed and analyzed as described for the other amino acids.

Calculation of digestibilities of CP and amino acids. The flow of CP and amino acids (AA; g/kg DMI) at the terminal ileum of the piglets were calculated using the respective CP and amino acid (aa) concentrations in digesta with chromic oxide (Cr) as indigestible marker, as follows:

$$(AA)_{flow} = (aa)_{digesta} \times [Cr_{diet} / Cr_{digesta}]. \quad \text{Eq. 1}$$

Apparent ileal digestibilities (AD), expressed as percentages, were calculated as follows:

$$(AD) = [(aa)_{diet} - (AA)_{flow} / (aa)_{diet}] \times 100. \quad \text{Eq. 2}$$

Statistical analysis. The results were subjected to analysis of variance according to Steel and Torrie (1980) using the model of Gill and Magee (1976) for four treatments with piglets (blocks), periods and diets as the main effects using the GLM procedures of SAS (1990). The model was as follows:

$$Y_{ijk} = \mu + Pig_i + Period_j + Diet_k + \varepsilon_{ijk}$$

where i = 12, j = 2 and k = 4. The experiment was completely balanced with diets adjusted for pig (block) effects and orthogonal to periods. Therefore, interactions were not included (Gill and Magee, 1976). Means of treatments and periods were compared using the Student Newman-Keuls' multiple range test procedure and the statistical significance level was claimed at $P < .05$.

Data pooled among the treatments for Periods 1 and 2 were compared using the Students' *t*-test according to Steel and Torrie (1980).

Results and Discussion

Performance of piglets prior to weaning. All piglets suckled the sow within 12 h after surgery (most by 8 h). Two of the piglets developed diarrhea 2 to 3 d after surgery and were given two or three 25 mL doses of an oral electrolyte maintenance solution (Pedialyte®, Ross Products Division, Abbott Laboratories Ltd., Saint-Laurent, QC) using a syringe and stomach tube. Three of the piglets were given 1 mL of Torbugesic (Butorphanol tartrate, 10 mg/mL; Ayerst Laboratories, Montreal, QC) as an analgesic on the day after surgery. It was not necessary at any time during the experiment to treat the piglets with antibiotics. The ADG of the piglets (corrected for weight of the cannula) between surgery and weaning was 170 ± 13 g/d compared to 233 g/d from birth to surgery. One of the piglets was lost because of twisting of the small intestine and one was crushed by the sow.

Analyses of diets. The CP and amino acid contents of the diets are presented in **Table 2**. The content of indispensable amino acids in all of the experimental diets were about .1 to .8 percentage units higher than NRC (1988) standards for 5 to 10 kg pigs. The incubation and protease spray processes did not affect the composition of soybean meal, as shown by similar CP and amino acid contents of the diets.

Post-weaning performance of the piglets. The piglets were healthy after weaning and no incidence of diarrhea was observed. There were no differences ($P > .05$) in ADG and feed conversion efficiency (**FCE**) between diets during the post-weaning period (**Table 3**). The ADG for all diets over the course of the experiment was 141 g/d which is less than the estimate of 250 g/d for piglets between 5 and 10 kg suggested by NRC (1988). The ADFI and FCE were approximately 25% lower than NRC (1988) standards. However, ADFI in the present study were comparable to values of 347 and 392 g/d for piglets fed diets containing untreated and protease-treated soybean meal, respectively, during the 3 wk period after weaning (Rooke et al., 1996). In their study, ADG and FCE were similar ($P > .05$) between the diets, although, piglets fed protease-treated soybean

meal had higher ($P < .01$) ADG (60 g/d) during the first wk after weaning than piglets fed untreated soybean meal. The lower performance of piglets in the present study compared to NRC (1988) standards, in part, can be explained by the fact that their average final BW was only 8.6 ± .4 kg. In this context, it should be mentioned that ADFI, ADG and FCE were lower ($P < .05$) during Period 1 (1 to 9 d after weaning) than in Period 2 (10 to 18 d after weaning). During the first 2 d after weaning most of the piglets

Table 2.
Chemical analyses and amino acid content[a] of the experimental diets[b].

	Control diets		Protease-treated diets	
Item	SBM	CI-SBM	PS-SBM	PI-SBM
DM (g/kg)	911	925	906	929
CP (g/kg)	228	229	224	222
GE (MJ/kg)	18.6	18.5	18.7	18.7
Amino acids (g/kg)				
Indispensable				
Arginine	13.3	13.5	12.8	12.7
Histidine	4.7	4.8	4.6	4.7
Isoleucine	8.8	9.3	8.7	9.0
Leucine	14.9	15.6	14.8	15.1
Lysine	13.5	14.1	13.7	13.7
Methionine	8.8	9.0	9.2	9.3
Phenylalanine	9.7	10.0	9.6	9.8
Threonine	8.5	9.0	8.4	8.6
Valine	9.2	9.7	9.1	9.4
Dispensable				
Alanine	8.0	8.4	7.8	8.1
Aspartate + Asparagine	23.0	24.0	22.8	23.2
Cysteine	6.0	6.0	6.0	6.0
Glutamate + Glutamine	36.2	37.6	36.1	36.4
Glycine	8.2	8.3	7.8	7.9
Serine	9.7	10.1	9.6	9.8
Tyrosine	5.2	6.1	5.1	5.8

[a] Dry mater basis.
[b] Refer to Table 1.

were eating less than 100 g/d but their consumption increased beginning on d 3. Performance during the first 9 d post-weaning was quite variable and some piglets lost weight, particularly those on the PS-SBM diet. Collection of digesta was not initiated until d 6 when all piglets consumed the diets at a daily rate greater than 3.4% of their BW (wt/wt) with the exception of one piglet on the PS-SBM diet that consumed the diet at a rate of only 2.0% of BW. During Period 2, all piglets consumed the diets at a daily rate of more than 5.5% of BW prior to the initiation of collection of digesta.

Apparent ileal digestibilities. The apparent ileal digestibilities of DM, CP and amino acids are presented in **Table 4**. There were no differences ($P > .05$) in CP and amino acid digestibilities among diets. The digestibilities of CP were 70.4, 72.4, 65.2 and 70.3% for the SBM, CI-SBM, PS-SBM and PI-SBM diets, respectively. Corresponding amino acid digestibilities ranged from 62.5, 67.5, 57.9 and 65.0% for alanine to 83.5, 83.4, 78.7 and 84.7% for arginine (Table 4). Alanine synthesized de novo by transamination of pyruvate is a primary oxidation substrate for the enterocytes (Hunt and Groff, 1990). Thus, the low digestibility of alanine probably results from the high metabolic turnover and sloughing of intestinal tissue in piglets. The digestibilities of threonine and glycine were also usually lower than of the other amino acids, ranging from 60.5 to 68.7 and 59.8 to 69.2%, respectively (Table 4). The low digestibilities of threonine and glycine are because of their relatively high content in mucin and bile salt conjugates, respectively (Fan et al., 1995). In addition, based on the specificity of endogenous proteases and peptidases, threonine is the last amino acid to be released by enzymatic hydrolysis in protein (Low, 1980). In contrast, the relatively high digestibilities of arginine, histidine and lysine indicate that these are first released from protein by proteases and peptidases (Low, 1980).

Table 3.
Initial BW, ADFI, ADG and feed conversion efficiency (**FCE**) of piglets fed the experimental diets[a].

	Control diets		Protease-treated diets		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[b]
Initial BW (kg)	6.4	6.3	6.5	6.4	.2
ADFI (g/d)	377.1	342.0	340.2	343.7	13.5
ADFI as % of BW	5.3	4.9	4.9	5.2	.3
ADG (g/d)	160.4	152.1	104.2	147.9	21.4
FCE (g gain/g feed)	.4	.4	.2	.4	.1

[a] Refer to Table 1.
[b] Standard error of the mean (n = 6).

It was expected that protease treatment would improve apparent ileal amino acid digestibilities as a result of increased solubility of protein and partial hydrolysis and inactivation of residual SBTI and, possibly, the protein component of lectins in soybean meal. The SBTI bind irreversibly to trypsin and chymotrypsin which impede their hydrolytic action, thereby, decreasing efficiency of protein digestion. Likewise, lectins

Table 4.
Apparent ileal digestibilities (%) of DM, CP and amino acids of the experimental diets[a].

	Control diets		Protease-treated diets		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[b]
DM	68.7	68.0	63.9	68.5	2.3
CP	70.4	72.4	65.2	70.3	4.1
Amino acids					
Indispensable					
Arginine	83.5	83.4	78.7	84.7	2.4
Histidine	80.1	82.1	76.9	80.5	2.8
Isoleucine	72.3	74.5	67.3	73.4	3.3
Leucine	72.9	74.2	67.1	74.0	3.4
Lysine	77.9	79.0	74.5	78.8	3.2
Methionine	72.9	74.6	82.6	74.1	3.4
Phenylalanine	73.8	75.2	68.2	74.4	3.3
Threonine	68.5	68.7	60.5	68.1	4.2
Valine	69.6	71.8	63.2	71.5	3.9
Dispensable					
Alanine	62.5	67.5	57.9	65.0	4.9
Aspartate + Asparagine	76.0	78.0	72.3	76.8	3.2
Cysteine	74.8	79.5	78.0	77.4	2.4
Glutamate + Glutamine	78.8	80.4	75.8	78.1	2.8
Glycine	69.1	69.2	59.8	67.4	4.4
Serine	75.6	76.4	69.6	76.9	3.3
Tyrosine	71.5	75.8	64.5	74.9	4.5

[a] Refer to Table 1.
[b] Standard error of the mean (n = 6).

are glycoproteins which have a strong binding affinity to terminal N-acetyl-D-galactosamine and D-galactose of glycoconjugates in the glycocalyx of intestinal epithelial cells and mucins secreted into the intestinal lumen (Schulze, 1994). The *in vitro* incubation of soybean meal under conditions similar to those in the present study (protease supplied at 1 μL/g of soybean meal) increased ($P < .05$) the proportion of soluble protein from 9.0 to 31.9% while the content of SBTI was reduced ($P < .05$) from 3.6 to 3.1 mg/kg (Caine et al., 1997). However, the increase in protein solubility and decrease in SBTI and likely lectin contents did not improve ($P > .05$) the ileal amino acid digestibilities in the piglets.

Apparent ileal amino acid digestibilities in growing pigs (35 kg initial BW), fed cornstarch-based soybean meal diets, reach a plateau when the threshold level of the amino acids in the diet are exceeded (Fan et al., 1994). These authors also pointed out that the threshold level of amino acids, at which apparent digestibilities do not increase, are characteristic of the specific diet studied. However, the similarities in diet composition between the aforementioned and present study makes a direct comparison possible. The contents of amino acids in all diets in the present study were higher than the threshold levels reported by Fan et al. (1994), ranging from .1 to .2 percentage units for the indispensable amino acids. Thus, protease treatment of soybean meal might not have improved amino acid digestibilities in the piglets for reasons that the threshold levels for amino acids were exceeded.

Effect of time after weaning on apparent digestibilities of amino acids. Apparent digestibility coefficients for experimental Periods 1 and 2 are presented in **Table 5**. With the exception of methionine, the apparent digestibilities of CP and amino acids during Period 1 (on d 7 after weaning) were lower ($P < .05$) than in Period 2 (on d 16 after weaning). It should be pointed out that the apparent digestibilities of methionine are not indicative of the different soybean meal diets. A proportion of methionine in the experimental diets was supplied as DL-methionine. The percentage unit increases ($P < .05$) in amino acid digestibilities from Periods 1 to 2 ranged from 8.6 for cysteine to 16.3 for threonine and tyrosine.

In part, growth depression in newly weaned piglets can be attributed to low apparent amino acid digestibilities. The apparent digestibilities for the indispensable amino acids during Period 1 were 5 to 16 percentage units lower than in Period 2. The digestibilities for Period 2 were similar to those summarized for soybean meal by Sauer and Ozimek (1986). Of the indispensable amino acids, the differences between Periods 1 and 2 were highest for threonine, phenylalanine and the branched-chain amino acids, ranging from 16.3 to 14.5 percentage units. The large increases in apparent digestibilities of the aforementioned amino acids in Period 2 suggest an improvement

in the recovery of endogenous protein. For instance, the higher digestibility of threonine in Period 2 may indicate a decrease in the loss of intestinal mucin. Aromatic and branched-chain amino acids are recognition sites for pancreatic proteases such as chymotrypsin and carboxypeptidase A (Hunt and Groff, 1990). Based on the known specificity of these pancreatic proteases the higher digestibilities of phenylalanine, tyrosine and the branched-chain amino acids during Period 2 is a further indication of an increase in the digestive and absorptive capacity of the piglets. This increased digestive and absorptive capacity after weaning seems to be an age and/or time dependent adaptation to a solid diet.

Table 5.
Effect of experimental Period on apparent ileal digestibilities (%) of CP and amino acids.

	Experimental Period[a]		
	One	Two	SEM[b]
CP	63.0	76.0	2.9
Amino acids			
Indispensable			
Arginine	77.9	87.2	1.7
Histidine	75.2	84.6	2.0
Isoleucine	64.6	79.1	2.4
Leucine	64.8	79.3	2.4
Lysine	72.4	82.6	2.3
Methionine	73.5	78.6	2.4
Phenylalanine	65.6	80.1	2.3
Threonine	63.5	79.8	3.2
Valine	61.3	76.7	2.7
Dispensable			
Alanine	55.5	71.0	3.4
Aspartate + Asparagine	69.3	82.3	2.3
Cysteine	73.1	81.7	1.7
Glutamate + Glutamine	72.3	84.2	2.0
Glycine	60.1	72.6	3.1
Serine	67.9	81.2	2.3
Tyrosine	63.5	79.8	3.2

[a] Differences between periods ($P < .05$) were found for CP and all amino acids except methionine.
[b] Standard error of the mean (n = 12)

The increase in digestibilities from 7 d to 16 d post-weaning suggests that the growth depression period of the piglets in the present study was relatively short, about 8 to 10 d. The improved digestive and absorptive capacity seems to correspond with an increase in feed intake (about 20%) from d 6 to 8 after weaning. In this context, Corring (1980) reported that a 5 to 7 d period is necessary for adaptation by the digestive enzyme secretions in pigs. This seems to be the case for newly weaned piglets adapting from milk to solid diets based on the effect of time after weaning on apparent ileal digestibilities of CP and amino acids.

In conclusion, neither spraying or incubation of soybean meal with protease improved the apparent digestibilities of CP and amino acids in newly-weaned piglets. As such, a greater proportion of soluble protein or lower content of residual SBTI (and likely lectins) in soybean meal were not the principal cause of lower digestibilities. Digestibilities were higher ($P < .05$) on d 16 versus d 7 after weaning. This difference indicates that a relatively short period is necessary for adaptation by the digestive enzyme secretions in piglets fed diets containing soybean meal.

Implications

Treatment of soybean meal with protease, either as a topical spray or by pre-feeding incubation, had no effect on apparent ileal digestibilities of amino acids in newly weaned piglets. The amino acid digestibilities were considerably higher on d 16 compared with d 7 after weaning which suggests an improvement in the digestive and absorptive capacity of the piglets during this time.

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Literature Cited

AOAC. 1990. Official Methods of Analysis. (15th Ed.). Association of Official Analytical Chemists. Arlington, VA.

Caine, W. R., M. W. A. Verstegen, W. C. Sauer, S. Tamminga, and H. Schulze. 1997. Effect of protease treatment of soybean meal on content of total soluble matter and crude protein and level of soybean trypsin inhibitors. Anim. Feed Sci. Technol., In press.

CCAC. 1980. Canadian Council on Animal Care. Guide to the Care and Use of Experimental Animals. Vol. 1 (with addendum). Ottawa, ON.

Corring, T. 1980. The adaptation of digestive enzymes to the diet: Its physiological significance. Reprod. Nutr. Develop. 20:1217-1235.

Fan, M. Z., W. C. Sauer, R. T. Hardin, and K. A. Lien. 1994. Determination of apparent ileal amino acid digestibility in pigs: effect of dietary amino acid level. J. Anim. Sci. 72:2851-2859.

Fan, M. Z., W. C. Sauer, and C. F. M. de Lange. 1995. Amino acid digestibility in soybean meal, extruded soybean and full-fat canola for early-weaned pigs. Anim. Feed Sci. Technol. 52:189-203.

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and faeces. Can. J. Anim. Sci. 59:631-634.

Gill, J. L., and W. T. Magee. 1976. Balanced two-period changeover designs for several treatments. J. Anim. Sci. 42:775-777.

Hunt, S. M., and J. L. Groff. 1990. Advanced Nutrition and Human Metabolism. West Publishing Co., St. Paul, MN.

Jones, B. N., and J. P. Gilligan. 1983. o-Phthaldialdehyde precolumn derivitization and reverse-phase high performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J. Chromatogr. 266:471-482.

Kik, M. J. L. 1991. Effect of lectins in legume seeds on the structure and function of the small intestinal mucosa. PhD. Thesis. State University of Utrecht, The Netherlands.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, J. D. Hancock, G. L. Allee, R. D. Goodband, and R. D. Klemm. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. J. Anim. Sci. 68:1790-1799.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. D. Klemm, D. W. Giesting, J. D. Hancock, G. L. Allee, and R. D. Goodband. 1991a. Measuring suitability of soybean products for early-weaned pigs with immunological criteria. J. Anim. Sci. 69:3299-3307.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. D. Klemm, and R. D. Goodband. 1991b. Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci. 69:4062-4069.

Low, A. G. 1980. Nutrient absorption in pigs. J. Sci. Food Agric. 31:1087-1130.

Makkink, C. A., and M. W. A. Verstegen. 1990. Pancreatic secretion in pigs. J. Anim. Physiol. a. Anim. Nutr. 64:190-208.

NRC. 1988. Nutrient Requirements of Swine (9th rev. Ed.). National Academy Press, Washington, D.C.

Rooke, J.A., H. Fraser, M. Shanks, and A. Morgan. 1996. The potential for improving soya-bean meal in diets for weaned piglets by protease treatment: comparison with other protein sources. Proceedings of the 112th meeting of the British Society of Animal Science, Scarborough, UK. Anim. Sci. 62:658.

Partridge, G., and H. Schulze. 1996. Effective application of feed enzymes in swine nutrition. Technical Symposium. p. 35-48. 17th Western Nutrition Conference, Edmonton, AB.

SAS. 1990. SAS/STAT® User's Guide (Release 6.08). SAS Inst. Inc., Cary, NC.

Sauer, W. C., and L. Ozimek. 1986. Digestibility of amino acids in swine: results and their practical applications. A review. Livest. Prod. Sci. 15:367-388.

Schulze, H. 1994. Endogenous ileal nitrogen losses in pigs - dietary factors. PhD. Thesis. Wageningen Agricultural University, The Netherlands.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Publishing Co., New York.

Tamminga, S., H. Schulze, J. van Bruchem, and J. Huisman. 1995. The nutritional significance of endogenous N-losses along the gastro-intestinal tract of farm animals. Arch. Anim. Nutr. 48:9-22.

van Leeuwen, P., D.J. van Kleef, G.J.M. van Kempen, J. Huisman, and M.W.A. Verstegen. 1991. The post valve T-caecum cannulation technique; an alternative method for chyme collection in pigs. J. Anim. Physiol. a. Anim. Nutr. 65:183-193.

4 Endogenous Recoveries and True Ileal Digestibilities of Amino Acids in Newly Weaned Piglets Fed Diets With Protease-Treated Soybean Meal

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ABSTRACT

Endogenous recoveries and true ileal digestibilities of amino acids were determined in piglets fed cornstarch-based diets with untreated or protease-treated soybean meal as protein source. Twelve piglets, fitted with a modified post valve T-cecum cannula on d 14, 15 and 16 after birth, were weaned on d 20 and assigned to one of four diets according to a two period balanced change-over design. Diets consisted of soybean meal untreated (SBM), incubated (1:2 wt/vol in distilled water adjusted to pH 4.5, for 16 h at 50°C; CI-SBM), sprayed with protease (1 µL/g of soybean meal; PS-SBM) and incubated with protease as described previously (1 µL/g of soybean meal; PI-SBM). Each period consisted of a 5 d adaptation to diets followed by collection of ileal digesta on d 6 and 7 to determine the apparent ileal amino acids digestibilities of the diets. On d 9, guanidinated meals were fed followed by a 24 h continuous collection of digesta. Recoveries of chromic oxide and dysprosium from the guanidinated meals were 96.0 ± .5 and 94.5 ± 1.1 %, respectively. Endogenous amino acid recoveries were similar ($P > .05$) for SBM, CI-SBM and PS-SBM but lower ($P < .05$) for PI-SBM. True digestibilities were also lower ($P < .05$) for PI-SBM compared with the other meals. Recoveries of endogenous branched-chain and aromatic amino acids were lower ($P < .05$) during Period 2 than Period 1, suggesting dietary change- and/or age-dependent adaptive increases in the secretions of pepsin and pancreatic proteases. In conclusion, protease treatment did not improve the true digestibilities of amino acids in soybean meal fed to newly weaned piglets.

Key Words: Piglets, Endogenous Amino Acids, Protease, Digestibilities

Introduction

Protein sources in diets for pigs should be evaluated by determining true ileal digestibilities of amino acids; apparent values corrected for recoveries of endogenous losses (Sauer and de Lange, 1992; Schulze, 1994). However, there are no reports of true ileal digestibilities of amino acids in feedstuffs fed to newly weaned piglets. The ratio of homoarginine to amino acids in guanidinated protein test meals and ileal digesta have been used to determine recoveries of endogenous amino acids from the distal ileum of growing pigs fed diets with low or high content of soybean trypsin inhibitors (**SBTI**; W.R. Caine, personal communication). This approach has not been used in digestibility studies with piglets soon after weaning.

Soybean meal, although a high quality protein source for growing pigs, does not have the efficacy of milk protein for newly weaned piglets. The presence of low levels of residual SBTI and lectins in soybean meal are thought to reduce protein digestibility in young piglets by inhibiting the digestive function of pancreatic enzymes and increasing losses of endogenous secretions such as mucins from the small intestine

(Tamminga et al., 1995). Trypsin inhibitors and lectins are protein-containing complexes that may be susceptible to proteolytic degradation and inactivation by protease enzymes. In this context, proteases are currently being developed by various companies for the feed industry. However, there were no differences ($P > .05$) in the apparent amino acid digestibilities in newly weaned piglets fed diets containing protease-treated and untreated soybean meal (Caine et al., 1997). The objective this study was to determine the effect of protease treatment of soybean meal on the endogenous recoveries and true ileal digestibilities of amino acids in piglets within 18 d after weaning at 20 d of age.

Experimental

Management of piglets and preparation of the experimental diets were previously described in detail by Caine et al. (1997). Sixteen baby piglets with an average birth weight of 1.8 ± .1 kg were fitted with a modified post valve T-cecum cannula (**mPVTC**) on d 14, 15 and 16 after birth. The average BW of the piglets prior to surgery was 5.3 ± .2 kg. The piglets were weaned on d 20 and transferred to individual metabolism crates (height; 85 cm, length; 70 cm and width; 65 cm) located in a room in which the temperature was maintained between 28 and 32°C. The average BW of piglets at weaning was 6.2 ± .3 kg. The twelve most vigorous piglets were selected for the study.

Experimental protocol. The experiment was carried out according to a two period changeover design for four treatments (Gill and Magee, 1976). The experimental diets were cornstarch-based with untreated or protease-treated (*Bacillus subtilis* subtilisin-protease, Finnfeeds International Ltd.) soybean meal, formulated to contain 20% CP. Four different forms of soybean meal (48% CP) from a single commercial source were prepared. Soybean meal which was untreated (**SBM**) or incubated (1:2, wt/vol in distilled water with pH adjusted to 4.5 with HCl and then heated for 16 h at 50°C; **CI-SBM**) were the two "Control diets". Soybean meal sprayed with protease (1 µL/g of soybean meal; **PS-SBM**) or incubated as described for CI-SBM, except that the water solution supplied protease (1 µL/g of soybean meal; **PI-SBM**) were the two "Protease-treated diets". Formulation of the guanidinated protein meals are presented in **Table 1**. The formulation of the meals were similar to the diets previously described by Caine et al. (1997). Each experimental period lasted 9 days. Feed was offered to the piglets in equal amounts at 8-h intervals at 0800, 1600 and 2400 h. On d 6 and 7 ileal digesta were collected for a total of 24 h to determine the apparent ileal digestibilities of amino acids (Caine et al., 1997). At 2400 h on d 8 the piglets were given a cornstarch-based

Table 1.
Formulation (%)[a] of the guanidinated[b] and casein enzymatic hydrolysate (CEH) meals.

	Control meals		Protease-treated meals		CEH meals
	SBM	Cl-SBM	PS-SBM	Pl-SBM	
Corn starch	29.0	29.6	28.0	30.2	55.2
Soybean meal	43.2	42.6	44.2	42.0	-
CEH[c]	-	-	-	-	23.4
Dextrose	10.0	10.0	10.0	10.0	10.0
Cellulose[d]	6.0	6.0	6.0	6.0	-
Canola oil	5.0	5.0	5.0	5.0	5.0
Biophos[e]	3.5	3.5	3.5	3.5	3.5
Vitamin-mineral premix[f]	2.0	2.0	2.0	2.0	2.0
DL-Methionine	.4	.4	.4	.4	.4
Fortified salt[g]	.3	.3	.3	.3	.3
Chromic oxide	.3	.3	.3	.3	-
Antibiotic[h]	.2	.2	.2	.2	.2
Dysprosium chloride	.1	.1	.1	.1	-

[a] As-fed basis.

[b] Soybean meal which was untreated (SBM) and incubated (1:2 wt/vol in distilled water at 50°C and pH 4.5; Cl-SBM) were the two "Control diets" or sprayed (PS-SBM) and incubated (Pl-SBM) with protease (1 µL/g of soybean meal) were the two "Protease-treated diets".

[c] Casein Enzymatic Hydrolysate. Sigma Chemical Company, St. Louis, MO.

[d] Solka-Floc; powdered cellulose. Supplied by James River Corp., Berlin, NH.

[e] Provided (g/kg): available phosphorus, 150 to 180; calcium, 240. Supplied by Continental Lime Ltd., Exshaw, AB.

[f] Provided the following (mg/kg of diet): retinyl palmitate, 4.54; cholecalciferol, .025; α-tocopheryl acetate, 80.0; menadione, 4.0; cyanocobalamin, .06; riboflavin, 24.0; niacin, 80.0; dl-pantothenate, 50.0; choline, 1200.0; d-biotin, .5; folacin, 3.2; thiamin, 6.0; ethoxyquin, 10.0; Fe, 300.0; Mn, 40.0; Zn, 240.0; Cu, 250.0; I, .4; Se, .3. Supplied by Hoffmann-LaRoche Ltd., Meadowpine Bvd., Mississauga, ON.

[g] Provided (g/kg): NaCl, 950; Na, 380; I, .15; Co, .05; Cu, 3.5; Mn, 10.0; Zn, 9.0; Se, .075. Supplied by Champion Feed Services Ltd., Westlock, AB.

[h] Veterinary LS-20 premix. Provided the following (g/kg of premix): Lincomycin hydrochloride, 22; Spectinomycin sulphate, 22; mineral oil USP, 10; soybean meal carrier, 946. Supplied by The Upjohn Company, Animal Health Division, Orangeville, ON.

casein enzymatic hydrolysate (**CEH**) meal (Table 1). On d 9 the piglets were fed two guanidinated meals at 0800 and 1600 h, respectively. Digesta were collected continuously for 24 h starting at 0800 h. A second CEH meal was given at 2400 h on d 9 during the collection period. The CEH meals were fed before and after the guanidinated protein meals to prevent contamination by nondigested dietary protein and chromic oxide (**Cr**) from the previous diets and to maintain normal physiological and nutritional conditions of the digestive tract. Protein in the CEH meals was assumed to be 100% digestible and it's amino acids completely absorbed. The CEH and guanidinated protein meals were fed at the same rate as the experimental diets (Caine et al., 1997). Starting 45 min before the collection of digesta, the contents within the cecum were collected and discarded. Digesta were collected into soft plastic bags (length: 10 cm; i. d.: 1.5 cm) containing 5 mL of a solution of formic acid (10%, vol/vol) that was added to each bag to minimize bacterial fermentation. Bags were changed at least every hour and the contents pooled within piglet and immediately frozen at -20°C. At the conclusion of the 24-h collection period the dietary treatments of the piglets were switched according to the experimental design and the second period repeated as previously described.

The experimental proposal and surgical procedures were reviewed and approved by the Animal Care Committee of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta and the Wageningen Institute of Animal Sciences at the Wageningen Agricultural University. The animals were cared for in accordance with the guidelines established by CCAC (1980).

Preparation of the guanidinated protein meals. Soybean meal was guanidinated using the procedure of Schmitz et al. (1991) and subsequently sprayed (as previously described) to supply protease at 1 µL/g of soybean meal and air-dried for the PS-SBM meals. The PI-SBM and CI-SBM meals were prepared by guanidinating soybean meal that was previously incubated in distilled water, 1:2, wt/vol, at 50°C and pH 4.5 with or without protease (1 µL/g of soybean meal), respectively. This approach was chosen because of the uncertainty of the chemical stability of guanidinated soybean meal during incubation. Guanidinated soybean meal was freeze-dried after washing to remove isomethylurea and then crushed through a 2.36-mm sieve. The guanidinated meals were formulated on a DM basis using two-thirds guanidinated and one-third unguanidinated meal with all other ingredients included at the same level as for the diets previously presented by Caine et al. (1997). Chromic oxide (.3 %) was included as marker for calculation of the flow and digestibility of amino acids. In addition, dysprosium chloride was also included (.116 g/kg, providing approximately 50 mg/kg of dysprosium;

Dy) as a marker that was distinctive to the guanidinated meals. The recovery of Dy was used to evaluate the flow of digesta from the ileal-cecal valve into the mPVTC cannula.

Chemical analyses. Samples of the guanidinated protein meals and digesta were freeze-dried and ground through a .5-mm mesh screen in a Wiley mill before analyses. Dry matter contents in the guanidinated meals and digesta were determined according to AOAC (1990). The N contents were measured with an Automated N Analyzer (FP-428 Nitrogen Determinator, Leco® Corporation, St. Joseph, MI). Analyses of Cr and Dy content were performed by Instrument Neutron Activation Analysis according to the procedure described by Kennelly et al. (1980). Samples of the meals and digesta, approximately 2 g, were packed into 1.5 mL vials and irradiated at the University of Alberta SLOWPOKE II nuclear reactor. After a decay period, samples were counted by measuring the 320.1 and 108.2 keV γ-rays emitted by the radionuclides ^{51}Cr ($T^{1/2}$ = 27.7 d) and ^{165m}Dy ($T^{1/2}$ = 1.258 min.).

Calculation of flow and digestibilities of amino acids. The conversion of lysine to homoarginine in the guanidinated soybean meal samples was calculated as follows:

Conversion (%) = [homoarginine / (lysine + homoarginine)] × 100.

Total, endogenous and exogenous flows of amino acids were calculated from the ratio of homoarginine to the individual amino acids in the guanidinated meals and digesta. True ileal amino acid digestibilities were determined from the difference between total and endogenous flows of amino acids.

Statistical analyses. Data were subjected to analysis of variance according to Steel and Torrie (1980) using the model of Gill and Magee (1976) for four treatments as described by Caine et al. (1997). Statistical analyses were carried out using the GLM procedures of SAS (1990). Means of treatments and periods were compared using the Student Newman Keuls' multiple range test procedure and the statistical significance level was claimed at $P < .05$.

Data pooled among the treatments for Periods 1 and 2 were compared using the Students' *t*-test according to Steel and Torrie (1980).

Results and Discussion

The piglets were healthy throughout the experiment and readily consumed the guanidinated meals at the same level of intake as the experimental diets (Caine et al., 1997). Postmortem examinations at the conclusion of the experiment revealed no intestinal abnormalities, although, there were minor adhesions in some of the piglets.

The ADFI of the guanidinated meals was lower ($P < .05$) during Period 1 (d 9 after weaning) than Period 2 (d 18 after weaning). The ADFI of the guanidinated SBM, CI-SBM, PS-SBM and PI-SBM meals were 31.6, 34.9, 31.5 and 34.7 g/kg BW (SEM: 1.4), respectively, during Period 1. In the same order for the meals, the ADFI were 42.5, 39.6, 38.6 and 42.6 (SEM: 1.9), during Period 2.

Table 2.
Chemical analyses and amino acid content (g/kg DMI) of the guanidinated[a] and casein enzymatic hydrolysate (CEH) meals.

	Control meals		Protease-treated meals		CEH meals
Item	SBM	CI-SBM	PS-SBM	PI-SBM	
CP	288	280	270	218	223
Amino acids					
Homoarginine	13.1	10.4	12.0	5.1	-
Indispensable					
Arginine	16.5	16.1	15.5	12.7	7.8
Histidine	6.4	6.0	7.5	4.4	5.9
Isoleucine	11.7	11.4	11.1	9.0	12.3
Leucine	19.8	19.4	19.0	15.7	20.9
Lysine	7.1	7.8	7.4	7.4	21.2
Methionine	9.3	9.6	9.6	9.0	13.8
Phenylalanine	12.6	12.4	12.2	10.2	10.8
Threonine	11.0	10.3	11.2	8.4	10.5
Valine	12.5	12.0	11.7	9.4	14.9
Dispensable					
Alanine	10.7	10.1	9.8	7.5	6.9
Aspartate + Asparagine	29.5	29.5	29.0	22.3	17.7
Cysteine	6.7	6.8	6.3	5.9	1.3
Glutamate + Glutamine	45.5	46.1	45.7	34.1	53.7
Glycine	10.7	9.6	10.0	7.7	4.2
Serine	12.9	12.8	12.4	9.7	13.0
Tyrosine	7.6	7.8	7.7	5.7	3.7

[a] See footnote b of Table 1.

Analyses of the guanidinated meals. The CP and amino acid contents of the guanidinated meals are presented in **Table 2**. Crude protein and amino acid contents were similar between the SBM, CI-SBM and PS-SBM meals. Corresponding contents for the PI-SBM meals were 15 to 25% lower. The content of amino acids was approximately 20% higher in the guanidinated meals compared with the diets (Caine et al., 1997). These increases in amino acid content likely resulted from the loss of soluble carbohydrates during the repeated washings to remove residual methylisourea. The average recovery of material from the batches of soybean meal that were guanidinated was 72% (DM basis). The CP and amino acid contents of the PI-SBM guanidinated meals and diets were similar.

Prior to the experiment, it was decided that the batches of soybean meal to be guanidinated for the CI-SBM and PI-SBM meals should first undergo incubation, then guanidination. This approach was taken for two reasons. Firstly, so that the batches of soybean meal that were guanidinated for the CI-SBM and PI-SBM meals were from the same sources of soybean meal used in the diets. Secondly, it was decided that guanidination should follow incubation because of the uncertainty of the chemical stability of homoarginine to form Maillard or other products. Maillard products may be formed under the pH and moisture conditions that occur during guanidination (Lingnert, 1990). This approach, however, resulted in significant losses of amino acids in the PI-SBM meals. The protease apparently digested a portion of the protein which was subsequently lost during washing and centrifugation to remove residual methylisourea. Moughan and Rutherfurd (1990) described a dialysis method for removing methylisourea from guanidinated gelatin for studies with rats. However, this technique is not appropriate for the preparation of larger quantities of material which are necessary for studies with pigs (P.J. Moughan; personal communication). Subsequent studies should consider an alternative method for removing residual methylisourea which is toxic. As was expected, the CP and amino acid contents of the CI-SBM meals were similar to the SBM and PS-SBM meals.

The lower amino acid content (especially homoarginine) of the PI-SBM guanidinated meals compared with the other meals (Table 2) indicates that a substantial proportion of lysine residues had been subjected to proteolysis by protease and lost during guanidination. For instance, trypsin is specific for cleavage of peptide linkages next to lysine and arginine (Hunt and Groff, 1990). Consequently, if peptide bonds next to lysine in guanidinated PI-SBM protein are preferentially hydrolyzed then many of the nearby amino acids will be selectively released from the smaller peptides,

Table 3.
Recoveries (%) of chromic oxide and dysprosium in ileal digesta.

	Control meals[a]		Protease-treated meals[a]		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[b]
Chromic oxide	95.6	95.4	97.1	96.0	.5
Dysprosium	94.1	95.0	93.5	95.3	1.1

[a] See footnote b of Table 1.

[b] Standard error of the mean (n = 6).

solubilized and lost during the multiple washings to remove the methylisourea. Therefore, the conversion of lysine to homoarginine in the PI-SBM guanidinated meals may not have occurred at random as a result of previous enzymatic hydrolysis by protease. The combined content of homoarginine and lysine in the guanidinated PI-SBM meals (Table 2) was 10% less than the lysine content in the respective diet (Caine et al., 1997). This difference seemed to be because of the lower content of homoarginine in the PI-SBM meals, which was less than half that in any of the other meals (Table 2). The combined contents of lysine and homoarginine in the SBM, CI-SBM and PS-SBM guanidinated meals were 33, 22 and 29% higher (Table 2) than the lysine contents in the respective diets (Caine et al., 1997).

The average conversions of lysine to homoarginine in the batches of soybean meal prepared for the SBM, CI-SBM, PS-SBM and PI-SBM meals were 83, 77, 81 and 64%, respectively. These conversion rates are comparable to values of 78 and 73% for full-fat soybeans reported by Marty et al. (1994) and Barth et al. (1993), respectively. Siriwan et al. (1994) suggested that uniform guanidination of lysine in a protein source is required if homoarginine is used as a marker for determining endogenous recoveries of amino acids. They reported a constant ratio of homoarginine to other amino acids in casein and isolated soybean protein during a 4-h enzymatic digestion period and concluded that the homoarginine residues were evenly distributed. In contrast, ratios of homoarginine to some of the amino acids changed in protein sources such as cottonseed meal, soybean meal, corn, wheat, sorghum and meat meal. Even distribution of homoarginine residues can be expected in relatively simple but not in complex proteins. It is unlikely that homoarginine to amino acid ratios will remain similar for heterogeneous proteins, many of which are conjugated with carbohydrates. Indeed, non-uniform distribution of homoarginine residues in guanidinated material might parallel

the digestibility pattern of the different types of proteins comprising a protein source. Nevertheless, endogenous recoveries of amino acids determined using guanidinated meals, in which apparent digestibilities are different from their respective diets, must be interpreted with caution because in these instances the composition and true digestibility of the protein is likely to have changed.

Recoveries of Chromic oxide and Dysprosium in digesta collected from the mPVTC cannula. The recoveries of the digestibility markers Cr and Dy are presented in **Table 3**. The intakes of the guanidinated meals which contained Cr and Dy were carefully measured for the evaluation of the mPVTC cannula. There were no differences ($P > .05$) in the recoveries of the digestibility markers between and within treatments. Recoveries (%) for Cr and Dy ranged from 95.4 to 97.1 and 93.5 to 95.3, respectively. Corresponding average recoveries were 96.0 (SE: .4) and 94.5% (SE: .9). These results are very close to the value of 99 % for Cr reported by van Leeuwen et al. (1991) in growing pigs fitted with post valve T-cecum cannulas fed corn-based groundnut and sunflower meal diets. The nearly complete recoveries of both digestibility markers confirms the proper placement of the base of the barrel of the cannula close to the ileal-cecal valve. The apparent direct flow of digesta from the ileum into the barrel of the cannula suggests that little bacterial fermentation occurred in the cecum prior to collection. Based on the recovery values of Cr and Dy, either marker would be suitable for determining amino acid digestibilities. The mPVTC cannula seems to be appropriate for measuring ileal digestibilities in young piglets.

Apparent ileal digestibilities. Apparent ileal amino acid digestibilities of the guanidinated meals are presented in **Table 4**. With the exception of homoarginine, lysine, alanine and glycine, which were lower ($P < .05$) for the PI-SBM meals, the digestibilities were similar ($P > .05$) between the meals. The lower apparent digestibilities for the PI-SBM meals, particularly homoarginine and lysine, are probably related to their lower content in the meals because of losses during guanidination as was previously discussed.

A number of authors have discussed the underlying assumptions that are made when guanidinated protein is used to determine true digestibility values (Hagemeister and Erbersdobler, 1985; Moughan and Rutherfurd, 1990; Barth et al., 1993; Marty et al., 1994; Siriwan et al., 1994). Fundamental to all these assumptions is the premise that the composition of guanidinated protein in the test meal is representative of the unguanidinated protein in the diet and that the respective apparent digestibilities of amino acids are similar. Apparent digestibilities of amino acids, including residual lysine plus homoarginine, for the SBM, CI-SBM and PI-SBM guanidinated meals were usually

similar ($P > .05$) to the values of their respective diets, which were previously reported by Caine et al. (1997). The digestibilities of residual lysine in the guanidinated meals were similar ($P > .05$) at 65.9, 67.1, 62.6 and 64.6% (SEM: 1.8) for SBM, CI-SBM, PS-SBM and PI-SBM, respectively, but lower ($P < .05$) then values for the diets previously reported by Caine et al. (1997). The latter was expected because the digestibility of

Table 4.
Apparent ileal amino acid digestibilities (%) of the guanidinated meals[a].

	Control meals		Protease-treated meals		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[b]
CP	75.9	74.0	73.0	68.5	2.4
Amino acids					
Homoarginine	89.8[c]	89.4[c]	92.3[c]	80.7[d]	.8
Indispensable					
Arginine	86.3	85.1	84.7	82.4	.5
Histidine	78.5	78.0	75.2	71.8	1.7
Isoleucine	77.4	77.1	76.2	73.3	1.8
Leucine	78.3	77.7	76.8	74.9	1.8
Lysine	81.3[c]	79.8[c]	81.0[c]	71.1[d]	1.2
Methionine	78.7	76.5	72.7	82.3	3.5
Phenylalanine	79.6	78.9	78.1	76.5	1.7
Threonine	73.4	68.8	70.6	62.0	2.6
Valine	75.2	74.0	72.6	68.9	2.1
Dispensable					
Alanine	72.4[c]	70.6[c]	69.0[c]	60.6[d]	2.1
Aspartate + Asparagine	80.1	78.9	80.2	75.2	1.4
Cysteine	83.1	75.9	74.5	75.4	3.6
Glutamate + Glutamine	79.9	79.6	81.4	75.9	1.7
Glycine	72.7[c]	66.4[cd]	71.0[c]	56.6[d]	3.3
Serine	77.8	76.8	76.1	71.1	1.8
Tyrosine	80.5	81.0	78.8	74.7	1.7

[a] See footnote b of Table 1.
[b] Standard error of the mean (n = 6).
[c,d] Means in the same row with different superscripts differ ($P < .05$).

residual lysine in the guanidinated meals would be lower than in the diets because of the disproportionate contribution of endogenous lysine at the distal ileum. In contrast, digestibilities of most of the amino acids were 5 to 13 percentage units higher for the PS-SBM meal compared with it's respective diet (Table 4). The reason for these differences was not apparent because the only difference between the PS-SBM and SBM meals was the spray application of protease.

Endogenous and exogenous recoveries of amino acids. The recoveries of endogenous amino acids are presented in **Table 5**. There were no differences ($P > .05$) in the total of the endogenous amino acids between the SBM, CI-SBM and PS-SBM meals which were 26.2, 23.6 and 32.2 g/kg DMI, respectively.The total endogenous amino acids of 8.1 g/kg DMI for pigs fed PI-SBM, however, was lower ($P < .05$) than for the other meals. Endogenous recoveries of the individual amino acids followed the same pattern as the total of the amino acids. Recoveries were proportionally highest for threonine, alanine and glycine.

The recoveries of lysine for the SBM, CI-SBM and PS-SBM meals were similar to the value of 1.33 g/kg DMI for a cornstarch-based soybean meal diet fed to growing pigs reported by Marty et al. (1994), based on the total ileal flow of lysine and the apparent (true) digestibility of homoarginine. Whereas, the endogenous recovery of lysine for the PI-SBM meals was only 38% of the value reported by Marty et al. (1994). Losses of amino acids, particularly homoarginine, during guanidination would explain the lower ($P < .05$) endogenous amino acid recoveries of the piglets fed PI-SBM.

The endogenous protein recoveries for the SBM, CI-SBM and PS-SBM meals are in the range of the average value for pigs of 25 g/kg DMI reported by Tamminga et al. (1995). The estimates are also in agreement with the value of 25.5 g/kg DMI reported by de Lange et al. (1990) who fed a cornstarch-based soybean meal diet and used the ^{15}N-isotope dilution technique. As expected, the estimates were higher than those for pigs determined with the conventional method in which protein-free diets were fed (Sauer et al., 1977; de Lange et al., 1989a,b). It is well recognized now that the use of protein-free diets will result in an underestimation of endogenous protein recovered from the ileum of pigs. Furthermore, the results from the present study did not include proline which represented about 40% of the total of the endogenous amino acids in pigs fed protein-free diets (Sauer et al., 1977). De Lange et al. (1989b) reported that proline accounted for 5 and 19% of the total endogenous amino acids in pigs fed protein-free diets and parenterally administered amino acids and saline, respectively.

Table 5.
The recoveries (g/kg DMI) of endogenous amino acids in ileal digesta.

	Control meals[a]		Protease-treated meals[a]		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[b]
Total of the amino acids	26.2[c]	23.6[c]	32.2[c]	8.1[d]	3.4
Indispensable					
Arginine	.5[cd]	.5[cd]	.9[c]	-.1[d]	.2
Histidine	1.3[c]	1.2[c]	1.2[c]	.5[d]	.1
Isoleucine	1.6[c]	1.3[c]	1.9[c]	.4[d]	.2
Leucine	1.5[c]	1.2[c]	1.9[c]	.3[d]	.2
Lysine	1.1[c]	1.0[c]	1.3[c]	.5[d]	.2
Methionine	.3	.3	.5	-.4	.2
Phenylalanine	1.4[c]	1.1[c]	1.7[c]	.2[d]	.2
Threonine	2.2[c]	2.1[c]	2.6[c]	1.0[d]	.3
Valine	1.9[c]	1.6[c]	2.4[c]	.6[d]	.2
Dispensable					
Alanine	2.3[c]	2.0[c]	2.8[c]	1.0[d]	.2
Aspartate + Asparagine	1.3[c]	1.1[c]	1.5[c]	.3[d]	.2
Cysteine	.9[cd]	1.4[cd]	2.1[c]	.3[d]	.4
Glutamate + Glutamine	1.3[c]	1.0[c]	1.3[c]	.2[d]	.2
Glycine	2.2[c]	2.4[c]	2.6[c]	1.2[d]	.3
Serine	1.6[c]	1.3[c]	1.9[c]	.5[d]	.2
Tyrosine	1.2[cd]	.9[d]	1.6[c]	.3[e]	.2

[a] See footnote b of Table 1.
[b] Standard error of the mean (n = 6).
[c,d,e] Means in the same row with different superscripts differ ($P < .05$).

Therefore, the total endogenous recoveries of amino acids for the piglets fed the SBM, CI-SBM and PS-SBM guanidinated meals are minimum estimates.

The exogenous recoveries of amino acids are presented in **Table 6**. The total of the exogenous amino acids were similar ($P > .05$) between the SBM and CI-SBM meals, 25.5 and 30.0 g/kg DMI, respectively. The corresponding value for the PS-SBM meals was lower ($P < .05$) at 22.1 g/kg DMI compared with the CI-SBM meal. Exogenous

recoveries of most of the amino acids were higher ($P < .05$) for the PI-SBM compared with the other meals, although, as was discussed previously these results should be interpreted with caution.

Table 6.
The recoveries (g/kg DMI) of exogenous amino acids in ileal digesta.

	Control meals[a]		Protease-treated meals[a]		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[b]
Total of the amino acids	25.5^{de}	30.0^{d}	22.1^{e}	41.7^{c}	1.6
Indispensable					
Arginine	1.8^{cd}	1.9^{cd}	1.4^{d}	2.3^{c}	.1
Histidine	.0^{d}	.1^{d}	.1^{d}	.8^{c}	.1
Isoleucine	1.0^{e}	1.4^{d}	.7^{f}	2.1^{c}	.1
Leucine	2.8^{d}	3.1^{cd}	2.5^{d}	3.7^{c}	.2
Lysine	2.7^{d}	2.7^{d}	2.4^{d}	3.1^{c}	.1
Methionine	.9^{d}	1.0^{d}	.6^{d}	1.5^{c}	.1
Phenylalanine	1.3^{de}	1.5^{d}	1.0^{e}	2.2^{c}	.1
Threonine	.8^{d}	1.1^{d}	.7^{d}	2.2^{c}	.1
Valine	1.2^{e}	1.5^{d}	.8^{f}	2.3^{c}	.1
Dispensable					
Alanine	.7^{d}	1.0^{d}	.2^{e}	2.0^{c}	.1
Aspartate + Asparagine	4.6	5.1	4.3	5.3	.3
Cysteine	.3^{d}	.2^{d}	-.5^{e}	1.2^{c}	.2
Glutamate + Glutamine	7.9	8.4	7.2	8.0	.6
Glycine	.7^{d}	.9^{d}	.3^{e}	2.1^{c}	.1
Serine	1.3^{e}	1.7^{d}	1.0^{e}	2.3^{c}	.1
Tyrosine	.3^{e}	.6^{d}	.0^{e}	1.1^{c}	.1

[a] See footnote b of Table 1.
[b] Standard error of the mean (n = 6).
[c,d,e,f] Means in the same row with different superscripts differ ($P < .05$).

True ileal digestibilities of amino acids. The true ileal digestibilities of amino acids for the guanidinated meals are presented in **Table 7**. Except for some amino acids which were lower ($P < .05$) for the CI-SBM meals, the digestibilities for the SBM, CI-SBM and PS-SBM meals were similar ($P > .05$). Compared with the other meals, the amino acid digestibilities in the PI-SBM meal were 6 to 16 percentage units lower ($P < .05$).

Table 7.
True ileal amino acid digestibilities (%) of the guanidinated meals[a].

	Control meals		Protease-treated meals		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[b]
Amino acids					
Indispensable					
Arginine	89.1[c]	87.9[c]	90.6[c]	81.7[d]	.8
Histidine	100.3[c]	97.8[c]	98.3[c]	82.0[d]	1.2
Isoleucine	91.3[c]	88.3[d]	93.6[c]	77.5[e]	.8
Leucine	85.8[c]	83.9[c]	86.6[c]	76.7[d]	1.0
Lysine	86.7[c]	85.3[c]	88.0[c]	75.0[d]	1.1
Methionine	90.6[c]	89.6[c]	93.4[c]	83.9[d]	1.1
Phenylalanine	90.2[cd]	87.7[d]	92.1[c]	78.6[e]	.8
Threonine	92.8[c]	89.7[d]	93.9[c]	73.4[e]	.9
Valine	90.5[c]	87.4[d]	92.9[c]	75.3[e]	.8
Dispensable					
Alanine	93.6[d]	90.0[e]	97.7[c]	74.1[f]	1.0
Aspartate + Asparagine	84.4[c]	82.6[c]	85.2[c]	76.4[d]	1.0
Cysteine	96.1[d]	96.7[d]	108.5[c]	79.9[e]	2.6
Glutamate + Glutamine	82.7[c]	81.8[c]	84.3[c]	76.6[d]	1.3
Glycine	93.5[cd]	91.3[d]	96.6[c]	72.5[e]	1.1
Serine	90.0[c]	87.1[d]	91.8[c]	76.1[e]	.8
Tyrosine	96.5[c]	92.1[d]	99.8[c]	80.0[e]	1.1

[a] See footnote b of Table 1.
[b] Standard error of the mean (n = 6).
[c,d,e,f] Means in the same row with different superscripts differ ($P < .05$).

The homoarginine technique proposed by Hagemeister and Erbersdobler (1985) assumes that the apparent digestibility of homoarginine provides an estimate of the true digestibility of lysine in guanidinated protein. Since the original report, several research groups have discussed the necessity of uniform conversion of lysine to homoarginine during guanidination (e. g., Moughan and Rutherfurd, 1990; Schmitz et al., 1991; Siriwan et al., 1994). In addition, there should be no change in the composition and apparent digestibilities of amino acids in the guanidinated protein. The results from the present study suggest that the aforementioned assumptions are not necessarily correct. The true ileal digestibilities of residual lysine in the SBM, CI-SBM, PS-SBM and PI-SBM guanidinated meals were 3, 4, 4 and 6 percentage units lower, respectively, than the corresponding values for the apparent digestibilities of homoarginine. Thus, the conversion of lysine to homoarginine did not precisely parallel the digestibility pattern of the differently treated soybean meals. In this context, true digestibilities were underestimated, especially for the PI-SBM meals.

The true digestibilities of amino acids in the SBM, CI-SBM and PS-SBM meals ranged from 82.6 to 85.2% for aspartate and asparagine to 97.8 to 100.3% for histidine. These values are similar to the true ileal digestibilities of growing pigs fed a cornstarch-based soybean meal diet determined using the ^{15}N-isotope dilution technique (de Lange et al. 1990) but slightly lower than values determined using a single guanidinated meal of soybean meal (Marty et al. 1994). The lower digestibilities for the CI-SBM and PI-SBM meals suggest that the incubation process (50°C at pH 4.5) may have reduced the digestibility of amino acids in the soybean meal.

Effect of time after weaning on endogenous recoveries and true ileal digestibilities of amino acids. Endogenous recoveries and true ileal digestibilities of amino acids for Periods 1 (d 9 after weaning) and 2 (d 18 after weaning) are presented in **Table 8**. The endogenous recoveries of isoleucine, leucine, phenylalanine, valine and tyrosine were higher ($P < .05$) during Period 1 than Period 2. There were no differences ($P > .05$) in true amino acid digestibilities between the periods with the exception of a decrease ($P < .05$) in tyrosine from 93.5 to 90.7% from Period 1 to 2.

The lower ($P < .05$) recoveries of the branched-chain and aromatic amino acids during Period 2 compared with 1 suggest that there was an increase in the secretion of pepsin and pancreatic proteolytic enzymes. Post-weaning intestinal and pancreatic secretions of enzymes in piglets increase with a change from milk to solid diets in an age and/or time-dependent manner (Corring, 1980; Makkink and Verstegen, 1990). Pepsin preferentially hydrolyzes peptide bonds adjacent to leucine or aromatic amino acids (Hunt and Groff, 1990). Carboxypeptidase A hydrolyzes aromatic and branched-

chain amino acids at the carboxyl end of peptides (Hunt and Groff, 1990). Therefore, an increase in the secretions of pepsin and carboxypeptidase A would augment the release of these amino acids in a free form to be readily absorbed and thereby reduce their recoveries at the terminal ileum. In addition, transport systems for amino acids along the digestive tract may have recovered from atrophy of the intestinal mucosa which occurs at weaning. Post-weaning damage to intestinal morphology, in particular a decrease in

Table 8.
Effect of experimental Period on endogenous recoveries (g/kg DMI) and true ileal digestibilities (%) of amino acids.

	Endogenous recoveries			True digestibilities		
Experimental Period:	One	Two	SEM[a]	One	Two	SEM[a]
Amino acids						
Indispensable						
Arginine	.5	.4	.1	87.4	87.2	.6
Histidine	1.2	.9	.1	95.4	93.9	.9
Isoleucine	1.6[b]	1.1[c]	.1	88.1	87.2	.6
Leucine	1.5[b]	1.0[c]	.1	82.7	83.9	.7
Lysine	1.1	.9	.2	83.9	83.6	.8
Methionine	.1	.3	.2	89.4	89.3	.8
Phenylalanine	1.4[b]	.8[c]	.1	87.4	86.9	.6
Threonine	2.2	1.7	.2	88.0	86.9	.7
Valine	1.9[b]	1.4[c]	.2	86.9	86.1	.6
Dispensable						
Alanine	2.3	1.8	.2	89.6	88.1	.7
Aspartate + Asparagine	1.1	.9	.1	82.0	82.3	.7
Cysteine	1.2	1.2	.3	96.2	94.4	1.8
Glutamate + Glutamine	1.0	.9	.2	81.4	81.2	.9
Glycine	2.2	2.0	.2	88.7	88.3	.8
Serine	1.5	1.1	.2	86.4	86.1	.6
Tyrosine	1.2[b]	.8[c]	.1	93.5[b]	90.7[c]	.8

[a] Standard error of the mean (n = 12).

[b,c] Means in the same row, within endogenous recoveries or true digestibilities, with different superscripts differ ($P < .05$).

villus height, greatly reduces the capacity of young pigs to digest protein sources of plant origin such as soybean meal (Li et al., 1990, 1991). However, it is unlikely that the recovered intestinal transport systems would facilitate improved absorption of the aromatic and branched-chain amino acids without a concomitant increase in the uptake of the other amino acids.

The relatively high true digestibilities of amino acids indicate that recently weaned piglets produced adequate gastrointestinal enzymes to digest dietary protein. In contrast, the decrease in ileal recoveries of the branched-chain and aromatic amino acids and increase in apparent ileal amino acid digestibilities previously reported by Caine et al. (1997) during Period 2 compared with Period 1 suggest an adaptive increase in the secretion of enzymes that reduced the losses of endogenous protein from the small intestine. The adaptation of enzyme secretions seems to have been dependent on age of the piglets and/or change from a milk to solid diet.

In conclusion, treatment of soybean meal with protease either via incubation (PI-SBM) or spraying (PS-SBM) did not affect the endogenous recoveries and true digestibilities of amino acids in the piglets.

Implications

Protease treatment supplied at 1 µL/g of soybean meal, as a topical spray, did not improve ($P > .05$) the true ileal digestibilities of amino acids in newly-weaned piglets. Lower endogenous recoveries of the branched-chain and aromatic amino acids from the piglets, during Period 2 (d 18 after weaning) compared with Period 1 (d 9 after weaning) suggests dietary change and/or age-dependent adaptive increase in secretion of pepsin and pancreatic proteases.

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Literature Cited

AOAC. 1990. Official Methods of Analysis. (15th Ed.). Association of Official Analytical Chemists. Arlington, VA.

Barth, C. A., B. Lunding, M. Schmitz, and H. Hagemeister. 1993. Soybean trypsin inhibitor(s) reduce absorption of exogenous and increase loss of endogenous protein in miniature pigs. J. Nutr. 123:2195-2200.

Caine, W. R. 1997. Personal communication. Department of Animal Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands.

Caine, W. R., W.C. Sauer, S. Tamminga, M.W.A. Verstegen, and H. Schulze. 1997. Apparent ileal digestibilities of amino acids in newly weaned piglets fed diets with protease-treated soybean meal. J. Anim. Sci., In press.

CCAC. 1980. Canadian Council on Animal Care. Guide to the Care and Use of Experimental Animals. Vol 1 (with addendum). Ottawa, ON.

Corring, T. 1980. The adaptation of digestive enzymes to the diet: Its physiological significance. Reprod. Nutr. Develop. 20:1217-1235.

de Lange, C. F. M., W. C. Sauer, R. Mosenthin, and W. B. Souffrant. 1989a. 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. 67:746-754.

de Lange, C. F. M., W. C. Sauer, and W. B. Souffrant. 1989b. The effect of 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. 67:755-762.

de Lange, C. F. M., W. B. Souffrant, and W. C. Sauer. 1990. Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N-isotope dilution technique. J. Anim. Sci. 68:409-418.

Gill, J. L., and W. T. Magee. 1976. Balanced two-period changeover designs for several treatments. J. Anim. Sci. 42:775-777.

Hagemeister, H., and H. Erbersdobler. 1985. Chemical labelling of dietary protein by transformation of lysine to homoarginine: a new technique to follow intestinal digestion and absorption. Proc. Nutr. Soc. 44:133A.

Hunt, S. M., and J. L. Groff. 1990. Advanced Nutrition and Human Metabolism. West Publishing Co. , St. Paul, MN.

Kennelly, J. J., F. X. Aherne, and M. J. Apps. 1980. Dysprosium as an inert marker for swine digestibility studies. Can. J. Anim. Sci. 60:441-446.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, J. D. Hancock, G. L. Allee, R. D. Goodband, and R. D. Klemm. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. J. Anim. Sci. 68:1790-1799.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. D. Klemm, D. W. Giesting, J. D. Hancock, G. L. Allee, and R. D. Goodband. 1991. Measuring suitability of soybean products for early-weaned pigs with immunological criteria. J. Anim. Sci. 69:3299-3307.

Lingnert, H. 1990. Development of the maillard reaction during food processing. In: P.A. Finot, H.U. Aeschbacher, R.F. Hurrell and R. Liardon (Ed.) The Maillard Reaction in Food Processing, Human Nutrition and Physiology. p 171. Birkhauser Verlag, Basel, Switzerland.

Makkink, C. A., and M. W. A. Verstegen. 1990. Pancreatic secretion in pigs. J. Anim. Physiol. a. Anim. Nutr. 64:190-208.

Marty, B. J., E. R. Chavez, and C. F. M. de Lange. 1994. Recovery of amino acids at the distal ileum for determining apparent and true ileal amino acid digestibilities in growing pigs fed various heat-processed full-fat soybean products. J. Anim. Sci. 72:2029-2037.

Moughan, P. J., and S. M. Rutherfurd. 1990. Endogenous flow of total lysine and other amino acids at the terminal ileum of protein- or peptide-fed rats: the chemical labelling of gelatin protein by transformation of lysine to homoarginine. J. Sci. Food Agric. 52:179-192.

Moughan, P. J. 1996. Personal communication. Monogastric Research Centre, Massey University, Palmerston North, New Zealand.

SAS. 1990. SAS/STAT® User's Guide (Release 6.08). SAS Inst. Inc., Cary, NC.

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. 57:775-784.

Sauer, W. C., and C. F. M. de Lange. 1992. Novel methods for determining protein and amino acid digestibilities in feedstuffs. In: S. Nissen (Ed.) Modern Methods in Protein Nutrition and Metabolism. p 87. Academic Press, San Diego, CA.

Schmitz, M., H. Hagemeister, and H. F. Erbersdobler. 1991. Homoarginine labeling is suitable for determination of protein absorption in miniature pigs. J. Nutr. 121:1575-1580.

Schulze, H. 1994. Endogenous ileal nitrogen losses in pigs - dietary factors. PhD. Thesis. Wageningen Agricultural University, The Netherlands.

Siriwan, P., W. L. Bryden, and E. F. Annison. 1994. Use of guanidinated dietary protein to measure losses of endogenous amino acids in poultry. Br. J. Nutr. 71:515-529.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Publishing Co., New York.

Tamminga, S., H. Schulze, J. van Bruchem, and J. Huisman. 1995. The nutritional significance of endogenous N-losses along the gastro-intestinal tract of farm animals. Arch. Anim. Nutr. 48:9-22.

van Leeuwen, P., D. van Kleef, G. J. M. van Kempen, J. Huisman, and M. W. A. Verstegen. 1991. The post valve T-caecum cannulation technique in pigs applicated to determine the digestibility of amino acid in maize, groundnut and sunflower meal. J. Anim. Physiol. a. Anim. Nutr. 65:183-193.

5 Bacterial Contributions To Total and Endogenous Recoveries of Nitrogen and Amino Acids In Ileal Digesta of Newly Weaned Piglets Fed Protease-Treated Soybean Meal

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Abstract

The bacterial contributions to total and endogenous recoveries of nitrogen (N) and amino acids in ileal digesta of newly weaned piglets were determined. Twelve piglets were fed cornstarch-based diets with soybean meal which was untreated (SBM), incubated (1:2 wt vol^{-1} in distilled water adjusted to pH 4.5, for 16 h at 50°C; CI-SBM), sprayed with protease (1 mL kg^{-1} of soybean meal; PS-SBM) and incubated, as previously described, with protease (1 mL kg^{-1} of soybean meal; PI-SBM). Ileal digesta were collected from the piglets, for a total of 24 h, on d 6 to 7 and then d 15 to 16 after weaning. Bacterial material was isolated from pooled subsamples of freeze-dried digesta from piglets within each dietary treatment. The content of diaminopimelic acid (DAPA) in the bacterial isolates and ileal digesta were used to calculate bacterial contributions. The contents of DAPA, N and amino acids in the bacterial isolates were not different ($P > 0.05$) among the diets. Bacterial N contributions were similar ($P > 0.05$); 17.1, 16.4, 14.6 and 21.0 g/100 g of total N in digesta of the piglets fed SBM, CI-SBM, PS-SBM and PI-SBM, respectively. Bacterial contributions of arginine, isoleucine, leucine, phenylalanine, valine and serine were higher ($P < 0.05$) in ileal digesta of the piglets fed PI-SBM. Contributions to endogenous recoveries of N and amino acids were similar ($P > 0.05$) among the diets. The bacterial contributions of N and all amino acids were lower ($P < 0.05$) on d 6 to 7 than d 15 to 16 after weaning. Differences ranged from 3.0 g/100 g of threonine to 9.1 g/100 g of cysteine. With the exception of arginine, lysine, methionine and glycine bacterial contributions to endogenous recoveries were lower ($P < 0.05$) on d 6 to 7 than d 15 to 16 after weaning. In conclusion, the bacterial contributions of N and amino acids in ileal digesta of newly weaned piglets increases after weaning.

Key Words: Piglets, Protease, Bacteria, DAPA, Endogenous Amino Acids

Introduction

Protein of non-dietary origin recovered from the ileum of pigs is considered to be an endogenous loss. The largest proportion of ileal protein recovered from pigs is from endogenous sources, mainly mucoproteins, pancreatic and intestinal enzymes, salivary, gastric and bile secretions and also sloughed epithelial cells (Souffrant, 1991; Tamminga et al., 1995). However, protein of bacterial origin also contributes to endogenous losses recovered from the ileum of pigs. Estimates of bacterial contributions of nitrogen (N) and amino acids in ileal digesta of growing pigs fed cereal-based diets ranges from 20 to 50 g/100 g when diaminopimelic acid (DAPA) is used as marker (e.g., Dierick et al., 1983; Poppe et al., 1983; Drochner, 1984; Dugan et al., 1994; Schulze et al., 1994). These estimates suggest that bacterial N and amino acids must necessarily constitute a large proportion of the endogenous protein recovered from the ileum of pigs.

At weaning, piglets often experience diarrhea and grow poorly while they adapt to plant protein-based diets. The utilization of plant protein by newly weaned piglets may be improved by pretreatment with protease. Rooke et al. (1996) reported an increase in daily gain of piglets, during the first 7 d after weaning at 21 d of age, when they were fed a cornstarch-based diet with soybean meal pretreated with protease as protein source. Protease pretreatment increases the crude protein solubility of soybean meal (Caine et al., 1997a). Nevertheless, neither apparent (Caine et al., 1997b) or true (Caine et al., 1997c) ileal digestibilities of amino acids were increased in newly weaned piglets fed protease-treated soybean meal. This suggests that bacteria in the small intestine of newly weaned piglets may have a modifying effect on the ileal recovery of both endogenous and dietary protein, in the same way that the microflora in the large intestine affect fecal digestibility values (Sauer and Ozimek, 1986). Estimates of bacterial contributions to total and endogenous recoveries of N and amino acids in ileal digesta of newly weaned piglets have not been previously reported.

The objectives of this study were twofold. First, to determine the effect of protease treatment of soybean meal on bacterial contributions to total and endogenous recoveries of N and amino acids from the terminal ileum of piglets within 16 d after weaning at 20 d of age. Secondly, to determine bacterial contributions at different times after weaning.

Experimental

Animals and diets

Management of piglets and preparation of the experimental diets were previously described by Caine et al. (1997b). Twelve piglets were fitted with a modified post valve T-cecum cannula on d 14, 15 and 16 after birth. The piglets were weaned on d 20 and assigned to one of four experimental diets. The average body weight of piglets at weaning was 6.2 ± 0.3 kg. The experimental diets were cornstarch-based with untreated or protease-treated (*Bacillus subtilis* subtilisin-protease; Finnfeeds International Ltd.) soybean meal as protein source, formulated to contain 32 g N kg^{-1}. Four different forms of soybean meal (76.7 g N kg^{-1}) were prepared. Soybean meal which was untreated (SBM) or incubated (1:2 wt vol^{-1} in distilled water with pH adjusted to 4.5 with HCl and then heated for 16 h at 50°C; CI-SBM) were the two "Control diets". Soybean meal sprayed with protease (1 mL kg^{-1} of soybean meal; PS-SBM) or incubated as described for CI-SBM except that the water solution contained protease (1 mL kg^{-1} of soybean meal; PI-SBM) were the two "Protease-treated diets". Canola oil was added so that the

Table 1.
Formulation[a] (g kg^{-1}) of the experimental diets[b].

	Control diets		Protease-treated diets	
	SBM	CI-SBM	PS-SBM	PI-SBM
Corn starch	291	297	281	303
Soybean meal[c]	432	426	442	420
Dextrose	100	100	100	100
Cellulose[d]	60	60	60	60
Canola oil	50	50	50	50
Biophos[e]	35	35	35	35
Vitamin-mineral premix[f]	20	20	20	20
DL-Methionine	4	4	4	4
Fortified salt[g]	3	3	3	3
Chromic oxide	3	3	3	3
Antibiotic[h]	2	2	2	2

[a] As-fed basis.

[b] Soybean meal which was untreated (SBM) and incubated (1:2, wt vol^{-1} in distilled water at 50°C and pH 4.5; CI-SBM) were the two "Control diets" or sprayed (PS-SBM) and incubated (PI-SBM) with *protease* (1 mL kg^{-1} of *soybean meal*) were the two "Protease-treated diets".

[c] Contained the following (g kg^{-1}): DM, 902.0; CP, 479.5; Ash, 59.9. The soybean trypsin inhibitors content was 3.66 mg kg^{-1} of soybean meal. Supplied by New-Life Feeds, Parrish and Helmbecker Ltd., Edmonton, AB.

[d] Solka-Floc; powdered cellulose. Supplied by James River Corp., Berlin, NH.

[e] Provided (g kg^{-1}): available phosphorus, 150 to 180; calcium, 240. Supplied by Continental Lime Ltd., Exshaw, AB.

[f] Provided the following (mg kg^{-1} of diet): retinyl palmitate, 4.54; cholecalciferol, 0.025; α-tocopheryl acetate, 80.0; menadione, 4.0; cyanocobalamin, 0.06; riboflavin, 24.0; niacin, 80.0; dl-pantothenate, 50.0; choline, 1200.0; d-biotin, 0.5; folacin, 3.2; thiamin, 6.0; ethoxyquin, 10.0; Fe, 300.0; Mn, 40.0; Zn, 240.0; Cu, 250.0; I, 0.4; Se, 0.3. Supplied by Hoffmann-LaRoche Ltd., Meadowpine Bvd., Mississauga, ON.

[g] Provided (g kg^{-1}): NaCl, 950; Na, 380; I, 0.15; Co, 0.05; Cu, 3.5; Mn, 10.0; Zn, 9.0; Se, 0.075. Supplied by Champion Feed Services Ltd., Westlock, AB.

[h] Veterinary LS-20 premix. Provided the following (g kg^{-1} of premix): Lincomycin hydrochloride, 22; Spectinomycin sulphate, 22; mineral oil USP, 10; soybean meal carrier, 946. Supplied by The Upjohn Company, Animal Health Division, Orangeville, ON.

diets exceeded National Research Council (NRC; 1988) standards for digestible energy. Vitamins, minerals and DL-methionine were also included in the diets to meet or exceed NRC (1988) standards. Chromic oxide (3 g kg^{-1}) was included as a digestibility marker. The composition of the experimental diets are presented in **Table 1**.

Experimental procedure

The experiment was carried out according to a two period balanced change-over design (Gill and Magee, 1976). Each experimental period lasted 9 d. Feed was offered to the piglets at 8-h intervals at 0800, 1600 and 2400 h. Each piglet was offered fresh feed at each meal. Remaining feed was removed and weighed just before the subsequent meal. Ileal digesta were collected for a total of 24 h in alternating 8-h periods: from 0800 to 1600 h on d 6 and 2400 to 0800 h and 1600 to 2400 h on d 7 of each experimental period. For 45 min prior to the start of each collection period ileal digesta were collected and discarded to clear the previous contents in the cecum. Digesta were collected into soft plastic bags (length: 10 cm; internal diameter. 1.5 cm) attached to the barrel of the cannula. A 5 mL solution of formic acid (10%, vol vol^{-1}) was added to each bag to reduce bacterial activity. Bags were changed at least every hour and immediately frozen at -20°C. Digesta were pooled within piglet and 24-h collection period and freeze-dried before analyses. Subsequently, bacterial material was isolated from two pooled subsamples of the freeze-dried digesta from piglets within each dietary treatment. Digesta (25 g) was reconstituted in 1 L of solution containing 8.5 g NaCl, 6.0 g $MgSO_4$ and 2 mL of Triton-X-100. Thereafter, bacterial material was isolated as described by Dugan et al. (1994).

The experimental proposal and surgical procedures were reviewed and approved by the Animal Care Committee of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta and the Wageningen Institute of Animal Sciences at the Wageningen Agricultural University. The animals were cared for in accordance with the guidelines established by the Canadian Council on Animal Care (1980).

Chemical analyses

The bacterial isolates were freeze-dried and ground in a mortar and pestle before analyses. Samples of digesta were freeze-dried and ground through a 0.5-mm mesh screen in a Wiley mill (Arther H. Thomas Co., Philadelphia, PA) before analyses. The dry matter content of samples were determined according to Association of Official Analytical Chemists (1990). The N content of the diets, digesta and bacterial isolates were measured with an Automated N Analyser (FP-428 Nitrogen Determinator, Leco®

Corporation, St. Joseph, MI). Content of amino acids in diets, digesta and bacterial isolates were analysed after acid hydrolysis (6M HCl; 24 h at 100°C) as *o*-phthaldialdehyde derivatives by High Performance Liquid Chromatography (HPLC) according to the procedure of Jones and Gilligan (1983) using a Varian 5000 HPLC system with a Varian 3300 autosampler and a Varian Fluorichrom detector (excitation 340 nm, emission 450 nm; Varian Canada Inc., Mississauga, ON). The content of amino acids were analysed in duplicate samples of the hydrolysates injected on a Supelcosil 3 micron LC-18 reverse phase column (4.6 × 150 mm; Supelco, Sigma-Aldrich Canada Ltd., Mississauga, ON) equipped with a Supelco LC-18 reverse phase 20 to 40 micron guard column (4.6 × 50 mm).The contents of DAPA were similarly analysed as *o*-phthaldialdehyde derivatives by HPLC according to the procedure of Dugan et al. (1994). The content of N, DAPA and amino acids of the experimental diets are presented in **Table 2**.

Calculation of bacterial contributions

The bacterial contributions of N and amino acids in ileal digesta were calculated from the contents of DAPA and the respective N or amino acid (AA) in the bacterial isolates and digesta, as follows:

$$[\,DAPA_{digesta} / DAPA_{bacteria}\,] \times [AA_{bacteria} / AA_{digesta}\,] \times 100\ g.$$

The DAPA content in the experimental diets (Table 2) were substracted from values determined in the digesta of the piglets before bacterial contributions were calculated.

Estimates of endogenous recoveries of amino acids in ileal digesta of the piglets were previously reported by Caine et al. (1997c) using guanidinated protein test meals. However, as was discussed by the authors, 15 to 25% of the amino acids in the guanidinated PI-SBM that was used to formulate the protein test meals for this diet were solubilized and lost during guanidination. This resulted in an underestimation of endogenous recoveries for the piglets fed PI-SBM test meals compared to test meals for the other diets. Nevertheless, it was expected that there should not have been a difference in endogenous recoveries for piglets fed PI-SBM than the other diets because of similar ($P > 0.05$) apparent ileal amino acid digestibilities (Caine et al., 1997b). In this respect, the amino acids that were lost during guanidination (difference between the content of amino acids in the PI-SBM diet and guanidinated protein test meals) were assumed to be soluble and, therefore, completely digestible. On this basis, endogenous recoveries for the PI-SBM-fed piglets were recalculated by including the amino acids lost during guanidination as part of the content of the guanidinated protein test meal. Lysine

lost during guanidination was assumed to be converted to homoarginine at the same level as was reported for the PI-SBM test meals (Caine et al., 1997c). The recalculated endogenous recoveries of amino acids for PI-SBM-fed piglets were usually similar ($P > 0.05$) to estimates for the other diets (see **Appendix 1**). The endogenous recoveries of N for the diets were estimated based on the N content of the amino acids reported by Caine et al. (1997c).

Statistical analyses

The results were subjected to analysis of variance according to Steel and Torrie (1980) using the model of Gill and Magee (1976) for four treatments with piglets (blocks), periods and diets as the main effects using the General Linear Model

Table 2.
Content[a] of nitrogen, diaminopimelic acid (DAPA) and amino acids of the experimental diets[b].

	Control diets		Protease-treated diets	
Item	SBM	CI-SBM	PS-SBM	PI-SBM
Nitrogen (g kg^{-1})	36.5	36.6	35.8	35.5
DAPA (mg kg^{-1})	52	53	42	43
Amino acids (g kg^{-1})				
Indispensable				
Arginine	13.3	13.5	12.8	12.7
Histidine	4.7	4.8	4.6	4.7
Isoleucine	8.8	9.3	8.7	9.0
Leucine	14.9	15.6	14.8	15.1
Lysine	13.5	14.1	13.7	13.7
Methionine	8.8	9.0	9.2	9.3
Phenylalanine	9.7	10.0	9.6	9.8
Threonine	8.5	9.0	8.4	8.6
Valine	9.2	9.7	9.1	9.4
Dispensable				
Alanine	8.0	8.4	7.8	8.1
Aspartate + Asparagine	23.0	24.0	22.8	23.2
Cysteine	6.0	6.0	6.0	6.0
Glutamate + Glutamine	36.2	37.6	36.1	36.4
Glycine	8.2	8.3	7.8	7.9
Serine	9.7	10.1	9.6	9.8
Tyrosine	5.2	6.1	5.1	5.8

[a] Dry matter basis.
[b] Refer to Table 1.

procedures of the Statistical Analysis System (1990). The model was as follows:

$$Y_{ijk} = \mu + \textbf{Piglets}_i + \textbf{Periods}_j + \textbf{Diets}_k + e_{ijk}$$

where i = 12, j = 2 and k = 4. The experiment was completely balanced with diets adjusted for piglet effects and orthogonal to periods. Therefore, interactions were not included (Gill and Magee, 1976). Means of treatments were compared using the Student Newman-Keuls' multiple range test procedure and the statistical significance level was claimed at $P < 0.05$.

Data pooled among the diets for d 6 to 7 and d 15 to 16 were compared using the Students' *t*-test according to Steel and Torrie (1980).

Results and Discussion

Content of DAPA, N and amino acids in bacterial isolates

Diaminopimelic acid is found in the peptidoglycan crosslinking units of the cell wall of gram-negative bacteria. Differences in diets, methods of collection and time of sampling and the predominant bacterial species collected in digesta will affect estimates using DAPA as marker; as reported in studies with ruminants (e.g., Dufva et al., 1982; Broderick and Merchen, 1992). The use of DAPA as a marker may overestimate bacterial N and amino acid contents if a relatively large proportion of bacterial fragments, particularly cell walls, instead of intact cells are collected for analysis. In the present study, the bacterial isolates were collected from digesta previously frozen and freeze-dried and, therefore, contained cell wall fragments from ruptured cells. However, the effect of cell wall enrichment on estimates of bacterial contributions was minimum because values were determined from the ratios of DAPA in the bacterial isolates and corresponding digesta samples.

The content of N, DAPA and amino acids in the bacterial isolates are presented in **Table 3**. There were no differences ($P > 0.05$) among the diets which resulted, in part, from the large variation between the bacterial isolates within the same treatment. Large variations in DAPA content in ileal digesta of pigs have been reported previously (Drochner, 1984; Dugan et al., 1994). As such, DAPA-N as a percentage of total N in the bacterial isolates were similar ($P > 0.05$); 0.40, 0.45, 0.50 and 0.32 (SE: 0.10) for SBM, CI-SBM, PS-SM and PI-SBM, respectively. These estimates are similar to the value (0.36) for a pure culture of *Escherichia coli* (Laplace et al., 1985) and the value (0.40) for a pig-cecum strain of *Clostridium butyricum* (Synge, 1953) but lower than

the mean values (ranging from 0.68 to 0.80) of nine species of rumen bacteria grown in medias containing different concentrations of fermentable carbohydrate (Dufva et al., 1982).

The average contents of glutamate + glutamine and aspartate + asparagine in the bacterial isolates, expressed as a percentage of the sixteen amino acids measured, were 13.4 and 11.3, respectively. These estimates were highest of the amino acids in the bacterial isolates and similar to corresponding values of 12.7 and 11.1 for a pure culture of *E. coli* (Laplace et al., 1985). The average contents of the other amino acids were also similar to the values for a pure culture of *E. coli*. Laplace et al. (1985) also reported similar values for bacteria isolated from the feces of pigs fed a cornstarch-based fishmeal diet.This suggests that *E. coli* was probably the predominant bacteria present in ileal digesta of the piglets, although, other enterobacteriaceae species are

Table 3.
Content[a] of nitrogen, diaminopimelic acid (DAPA) and amino acids of bacterial material isolated from ileal digesta of newly weaned piglets fed the experimental diets[b].

	Control diets		Protease-treated diets		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[c]
Nitrogen (g kg^{-1})	64.0	54.7	51.3	49.9	6.8
DAPA (g kg^{-1})	1.9	1.8	1.8	1.1	0.6
Amino acids (g kg^{-1})					
Indispensable					
Arginine	7.7	7.2	6.5	6.8	0.6
Histidine	3.3	3.0	2.7	2.8	0.3
Isoleucine	9.4	8.8	8.3	8.5	0.7
Leucine	13.5	12.9	11.9	12.6	1.0
Lysine	10.3	9.3	8.6	8.3	1.1
Methionine	5.8	5.2	5.2	4.8	0.6
Phenylalanine	8.5	8.0	7.3	7.8	0.7
Threonine	7.7	7.2	6.7	6.9	0.6
Valine	10.5	9.7	9.2	9.3	0.9
Dispensable					
Alanine	9.5	9.1	8.6	8.4	0.8
Aspartate + Asparagine	17.2	15.7	14.2	14.7	1.6
Cysteine	5.8	5.5	4.9	5.2	0.5
Glutamate + Glutamine	20.3	18.9	16.4	17.9	1.8
Glycine	8.3	7.8	7.7	7.1	0.7
Serine	6.2	5.9	5.1	5.6	0.5
Tyrosine	5.6	5.3	4.7	4.9	0.5

[a] Dry matter basis.
[b] Refer to Table 1.
[c] Standard error of the mean (n = 2).

also likely to have similar amino acid profiles. It should also be mentioned that antibiotics (Lincomycin hydrochloride and Spectinomycin sulphate) were included in the diets fed to the piglets. These antibiotics are active against gram-positive and coliform pathogens such as *Staphylococcus*, *Streptococcus*, *Clostridium tetani*, *Clostridium welchii* and some strains of *E. coli* (Huber, 1982). In this case, the bacterial microflora in the ileal digesta of these piglets fed semi-purified diets would be different than the enterobacteriaceae population in growing pigs fed commercial diets. Dierick et al. (1986) reported that the dominant enterobacteriaceae in the small intestine of growing pigs supplemented with Virginiamycin and Spiramycin were *E. coli*, *lactobacillus acidophilus*, *lactobacillus fermenti* and *streptococcus faecalis*. Haemolytic *E. coli* and *lactobacilli* and *streptococci* were the predominant enterobacteriaceae in the small intestine of piglets with postweaning diarrhea or edema (McAllister et al., 1979).

Bacterial contributions to total recoveries

The contribution of bacterial N and amino acids to total recoveries in ileal digesta are presented in **Table 4**. Bacterial contributions to total N recoveries in ileal digesta of the piglets were similar ($P > 0.05$) among the treatments and higher than that of the individual amino acids. However, contributions to total N would have been overestimated with respect to amino acid N because of the high concentration of nucleic acids in bacteria (Broderick and Merchen, 1992). The bacterial contribution of arginine, isoleucine, leucine, phenylalanine, valine and serine was higher ($P < 0.05$) in piglets fed PI-SBM than the other diets. These increases ranged from 5.3 g/100 g of serine to 7.8 g/100 g of arginine. The higher bacterial contribution of the branched-chain amino acids and phenylalanine to total recoveries in ileal digesta of the PI-SBM-fed piglets tends to agree with the data of Laplace et al. (1985) for bacteria isolated from the feces of pigs fed a cornstarch-based fishmeal diet. These results suggest a greater enterobacteriaceae population in the small intestine of the piglets fed PI-SBM, perhaps as a result of an increased supply of soluble dietary protein. The pretreatment of soybean meal with protease increases the proportion of soluble protein (Caine et al., 1997a). This was expected to improve the digestion and absorption of the dietary protein, but neither apparent (Caine et al., 1997b) or true (Caine et al., 1997c) ileal amino acid digestibilities were increased ($P > 0.05$) in piglets fed PI-SBM. The protease pretreatment did, however, apparently increase the availability of the soybean meal protein for assimilation and utilization by the intestinal bacteria. Ostensibly, the diets fed to the piglets probably limited the growth of the enterobacteriaceae. The main energy and carbon skeleton source in the experimental diets was cornstarch that is highly

digestible and, therefore, was probably not readily available for the metabolic activity of the bacteria in the lower regions of the small intestine. As such, bacteria in the small intestine of the PI-SBM-fed piglets probably utilized the protease-treated soybean meal to proliferate and grow to a greater extent than was possible in piglets fed the other diets. On the other hand, protease pretreatment of soybean meal may have encouraged a shift in the predominant bacterial species present in the small intestine of the

Table 4.
Bacterial contributions (g/100 g) to total recoveries of nitrogen and amino acids in ileal digesta of newly weaned piglets fed the experimental diets[a].

	Control diets		*Protease-treated diets*		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[b]
Nitrogen	17.1	16.4	14.6	21.0	2.1
Amino acids					
Indispensable					
Arginine	10.1[d]	10.2[d]	8.8[d]	16.6[c]	1.4
Histidine	10.0	10.9	8.8	13.7	1.2
Isoleucine	10.9[d]	11.5[d]	10.1[d]	16.4[c]	1.3
Leucine	9.5[d]	10.1[d]	8.8[d]	15.0[c]	1.3
Lysine	10.0	9.7	8.7	12.9	1.3
Methionine	16.4	16.6	20.9	18.0	2.8
Phenylalanine	9.5[d]	10.1[d]	8.7[d]	14.5[c]	1.2
Threonine	8.2	7.9	7.2	11.5	0.9
Valine	10.6[d]	10.9[d]	9.6[d]	16.1[c]	1.3
Dispensable					
Alanine	9.3	9.9	9.1	13.4	1.2
Aspartate + Asparagine	8.9	9.3	8.3	12.7	1.2
Cysteine	13.1	16.1	16.5	19.0	2.6
Glutamate + Glutamine	7.8	8.4	7.4	10.4	1.2
Glycine	9.5	9.0	9.0	12.6	1.3
Serine	7.5[d]	7.8[d]	6.4[d]	11.7[c]	0.9
Tyrosine	10.6	11.1	9.9	15.5	1.5

[a] Refer to Table 1.
[b] Standard error of the mean (n = 6).
[c,d] Means in the same row with different superscripts differ ($P < 0.05$).

PI-SBM-fed piglets. The DAPA-N as a percentage of total N of different bacterial species varies from 0 in *Streptococcus bovis* to 1.6 in *Clostridium welchii* (e.g. Synge, 1953; Dufva et al., 1982). However, as was previously discussed, the amino acid composition of the bacterial isolates for the piglets fed PI-SBM and the other diets were indicative of a predominant population of *E. coli*.

Bacterial contribution to endogenous recoveries

The contributions of bacterial N and amino acids to endogenous recoveries in ileal digesta of the piglets are presented in **Table 5**. Contributions to endogenous recoveries followed a similar pattern as with the total recoveries, although, there were large variations in estimates within and among the diets. In this context, estimates for the

Table 5.

Bacterial contributions (g/100 g) to endogenous recoveries[a] of nitrogen and amino acids in ileal digesta of newly weaned piglets fed the experimental diets[b].

	Control diets		Protease-treated diets		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[c]
Nitrogen	39.7	46.5	30.0	36.9	6.5
Amino acids					
Indispensable					
Arginine	60.6	40.6	29.8	48.3	8.0
Histidine	10.1	15.3	11.1	9.8	2.8
Isoleucine	18.0	26.9	15.2	22.6	4.0
Leucine	27.6	40.7	22.9	35.4	5.9
Lysine	34.8	39.6	26.2	42.0	7.1
Methionine	47.9	27.8	38.7	22.8	10.1
Phenylalanine	18.8	27.3	15.5	23.1	3.8
Threonine	11.4	12.4	9.7	13.2	1.5
Valine	17.3	22.9	13.9	21.6	3.0
Dispensable					
Alanine	12.1	15.6	10.3	14.3	2.0
Aspartate + Asparagine	43.3	61.1	35.5	48.4	9.8
Cysteine	16.9	19.6	28.3	19.0	4.1
Glutamate + Glutamine	60.5	54.9	58.2	66.4	7.6
Glycine	12.7	12.2	10.8	12.9	1.7
Serine	14.2	19.7	10.6	17.1	2.8
Tyrosine	13.1	23.4	11.7	14.3	4.1

[a] Endogenous amino acid recoveries were previously reported by Caine et al. (1997c). Endogenous nitrogen was estimated based on the nitrogen content of the amino acids reported.

[b] Refer to Table 1.

[c] Standard error of the mean (n = 6).

piglets fed PI-SBM were not different ($P > 0.05$) than for the other diets. The bacterial contributions of N to endogenous recoveries were higher than expected because the estimates of total recoveries were lower than values previously reported for older pigs; as reviewed by Dugan et al. (1994). In a review of the literature, Souffrant (1991) suggested that bacterial protein, when included as part of the endogenous ileal losses, may be the largest single contributor to endogenous protein recoveries. Bacterial N contributions as high as 90 g/100 g of N in ileal digesta of pigs fed protein-free diets have been reported [Ahrens and Kaufmann (1985), cited by Souffrant (1991)]. In the present study, the contribution of bacterial N ranged from 30.0 to 46.5 g/100 g of endogenous N in ileal digesta of the piglets fed the different diets. The bacterial contributions of amino acids to endogenous recoveries ranged from 9.7 g/100 g of threonine for PS-SBM-fed piglets to 66.4 g/100g of glutamate + glutamine for PI-SBM-fed piglets. The large variation between the bacterial contributions of the individual amino acids to endogenous recoveries suggests that the enterobacteriaceae modify the composition of endogenous ileal protein. For instance, the bacterial contribution of arginine and methionine were high relative to their content in the bacterial isolates. These amino acids have a low content in endogenous secretions such as intestinal mucus (Lien et al., 1997) and pancreatic juice (Corring and Jung, 1972). The enterobacteriaceae, therefore, increased the proportion of these amino acids in endogenous protein relative to contributions from endogenous secretions. In contrast, bacterial contributions were low for those amino acids which constitute a large part of intestinal mucoprotein or bile, such as cysteine and threonine or glycine, respectively. In this context, the amino acid composition of endogenous protein recovered at the terminal ileum of the piglets was dependent on the enterobacteriaceae. The effect of bacterial contributions to the amino acid composition of endogenous protein recovered from older pigs, fed cereal-based diets, is likely to be even more dependent on the population and diversity of species of bacteria in the small intestine.

The limited availability of dietary nutrients, as was previously discussed, may have caused a shift to increased scavenging by the enterobacteriaceae of endogenous protein and energy sources such as the epithelial tissue and secretions of the intestinal mucosa. According to McAllister et al. (1979), haemolytic *E. coli* were the only bacteria to markedly proliferate in the intestinal lumen of piglets after weaning. They reported the highest cell biomass in the distal regions of the small intestine. Facultative anaerobes such as *E. coli* can also control populations of other bacteria. This suggests that the enterobacteriaceae act as a N-sink by utilizing both dietary and endogenous protein for growth, making it unavailable for digestion and absorption by the pig. In part, this nullified the effect of protease pretreatment of the soybean meal as was indicated by the similar ($P > 0.05$) apparent ileal digestibilities among the diets (Caine et al. 1997b).

Effect of time after weaning

The effect of time after weaning on bacterial contributions of N and amino acids to total and endogenous recoveries in ileal digesta of the piglets are presented in **Table 6.** The contributions of N and all amino acids to total recoveries were lower ($P < 0.05$) on d 6 to 7 than d 15 to 16 after weaning. The bacterial N contributions for both experimental periods were lower than estimates previously reported for pigs fed cereal-

Table 6.
Effect of days after weaning on bacterial contributions (g/100 g) to total and endogenous recoveries of nitrogen and amino acids in ileal digesta of newly weaned piglets.

	Total Recoveries[a]			Endogenous recoveries		
Days after weaning:	6 to 7	15 to 16	SEM[b]	6 to 7	15 to 16	SEM[b]
Nitrogen	14.3	20.2	1.5	30.3[d]	46.3[c]	4.6
Amino acids						
Indispensable						
Arginine	8.7	14.1	1.1	40.6	49.9	5.6
Histidine	8.8	12.9	0.9	8.2[d]	15.0[c]	2.0
Isoleucine	9.7	14.8	1.0	14.9[d]	26.5[c]	2.8
Leucine	8.5	13.2	0.9	22.4[d]	40.9[c]	4.2
Lysine	8.4	12.1	1.0	28.7	42.6	5.0
Methionine	14.1	21.8	2.0	34.4	34.6	7.1
Phenylalanine	8.3	13.0	0.9	14.6[d]	27.7[c]	2.7
Threonine	7.2	10.2	0.7	9.4[d]	13.9[c]	1.1
Valine	9.5	14.2	0.9	14.3[d]	23.6[c]	2.1
Dispensable						
Alanine	8.6	12.2	0.8	10.5[d]	15.7[c]	1.4
Aspartate + Asparagine	7.7	12.0	0.8	34.5[d]	59.6[c]	6.9
Cysteine	11.6	20.7	1.8	15.1[d]	26.9[c]	2.9
Glutamate + Glutamine	6.5	10.5	0.9	50.1[d]	71.7[c]	5.4
Glycine	8.4	11.6	0.9	10.2	14.1	1.3
Serine	6.6	10.1	0.7	11.4[d]	19.4[c]	1.9
Tyrosine	9.1	14.4	1.0	10.3[d]	20.9[c]	2.9

[a] Differences between days after weaning ($P < 0.05$) were found for bacterial contributions to total recoveries of nitrogen and all amino acids.

[b] Standard error of the mean (n = 12).

[c,d] Means in the same row, within endogenous recoveries, with different superscripts differ ($P < 0.05$).

based diets (e.g. Dierick et al., 1983; Poppe et al., 1983; Drochner, 1984; Dugan et al., 1994). Differences in bacterial contributions of amino acids to total recoveries on d 6 to 7 than d 15 to 16 after weaning ranged from 3.0 g/100 g of threonine to 9.1 g/100 g of cysteine. These results would explain the improved weight gain only during the first 7 d after weaning in piglets fed protease-treated versus untreated soybean meal reported by Rooke et al. (1996). An increasing enterobacteriaceae population in the small intestine of the piglets after weaning may have compromised the potential beneficial effect of protease pretreatment of soybean meal.

Table 7.

Effect of days after weaning on recoveries[a] of nitrogen and amino acids from endogenous secretions in ileal digesta of newly weaned piglets.

	Endogenous Secretions		
Days after weaning:	6 to 7	15 to 16	SEM[b]
Nitrogen (g kg^{-1})	3.38[c]	2.18[d]	0.36
Amino acids (g kg^{-1})			
Indispensable			
Arginine	0.54	0.31	0.12
Histidine	1.34	1.11	0.09
Isoleucine	1.62[c]	1.06[d]	0.14
Leucine	1.46[c]	0.80[d]	0.13
Lysine	0.89	0.63	0.12
Methionine	0.41	0.56	0.34
Phenylalanine	1.46[c]	0.84[d]	0.12
Threonine	2.39	1.91	0.21
Valine	1.97[c]	1.37[d]	0.16
Dispensable			
Alanine	2.41	1.89	0.17
Aspartate + Asparagine	0.96[c]	0.55[d]	0.11
Cysteine	1.22	1.18	0.25
Glutamate + Glutamine	0.72	0.38	0.10
Glycine	2.48	2.18	0.24
Serine	1.67	1.23	0.15
Tyrosine	1.38[c]	0.91[d]	0.13

[a] Recoveries from endogenous secretions were determined, on a dry matter intake basis, from previously reported endogenous recoveries (Caine et al. 1997c) minus bacterial contributions (see Table 6).

[b] Standard error of the mean (n = 12).

[c,d] Means in the same row with different superscripts differ ($P < 0.05$).

The modifying effect of a growing enterobacteriaceae population in the small intestine of the piglets after weaning is shown by determining the N and amino acids contribution of endogenous secretions (endogenous recoveries minus bacterial contributions) presented in **Table 7**. In contrast to bacterial contributions, recoveries of N, phenylalanine, aspartate + asparagine, tyrosine and the branched-chain amino acids from endogenous secretions were lower ($P < 0.05$) on d 15 to 16 than d 6 to 7. This would be expected as digestive and absorptive capacity of the piglets improved with time after weaning (Caine et al., 1997b). Interestingly, the estimates for N recoveries from endogenous secretions are within the range of 1.15 to 3.67 g N kg^{-1} dry matter intake for pigs fed N-free diets (Seve and Leterme, 1997). In fact, the endogenous secretion of 2.18 g N kg^{-1} dry matter intake for d 15 to 16 is close to an average value of 2.34 g N kg^{-1} dry matter intake compiled from a large number of studies using N-free diets [Bastianelli (1996), cited by Seve and Leterme (1997)]. Wunsche et al. (1987) also reported a similar value of 2.2 g N kg^{-1} dry matter intake. The content of the amino acids recovered from endogenous secretions, in the present study, were different than the estimates for pigs fed N-free diets as summarized by Wunsche et al. (1987). These results clearly indicate that bacteria in the small intestine of pigs assimilate and change the amino acid composition of a large portion of endogenous protein. Furthermore, the enterobacteriaceae population in the small intestine of newly weaned piglets increases with time after weaning.

In conclusion, incubation of soybean meal with protease increased ($P < 0.05$) the bacterial contribution of arginine, phenylalanine, serine and the branched-chain amino acids to total recoveries in ileal digesta of newly weaned piglets. The bacterial contributions to total and endogenous recoveries of N and amino acids in ileal digesta increases ($P < 0.05$) with time after weaning. In this respect, the enterobacteriaceae acted as a N-sink by assimilating available protein, thereby, making it unavailable for absorption by the piglets. Further studies to delineate the role of bacteria in digestion and absorption of dietary and endogenous protein are warranted.

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Literature Cited

Association of Official Analytical Chemists. 1990. Official Methods of Analysis. (15th Ed.). AOAC. Arlington, VA.

Broderick, G.A. and Merchen, N.R., 1992. Markers for quantifying microbial protein synthesis in the rumen. J. Dairy Sci., 75, 2618-2632.

Caine, W.R., Verstegen, M.W.A., Sauer, W.C., Tamminga, S. and Schulze, H., 1997a. Effect of protease treatment of soybean meal on content of total soluble matter and crude protein and level of soybean trypsin inhibitors. Anim. Feed Sci. Technol., In press.

Caine, W. R., Sauer, W.C., Tamminga, S., Verstegen, M.W.A. and Schulze, H., 1997b. Apparent ileal digestibilities of amino acids in newly weaned piglets fed diets with protease-treated soybean meal. J. Anim. Sci. In press.

Caine, W.R., Tamminga, S., Verstegen, M.W.A., Sauer, W.C. and Schulze, H., 1997c. Endogenous recoveries and true ileal digestibilities of amino acids in newly weaned piglets fed diets with protease-treated soybean meal. J. Anim. Sci. In press.

Canadian Council on Animal Care. 1980. Guide to the Care and Use of Experimental Animals. Vol. 1 (with addendum). Ottawa, ON.

Corring, T. and Jung, J., 1972. The amino acid composition of pig pancreatic juice. Nutr. Rep. Int., 6, 187-190.

Dierick, N.A., Decuypere, J.A., Vervaeke, I.J. and Hendrickx, H.K., 1983. Digestion ileale et fecale de la matiere seche, des proteines et acides amines de queques rations conventionnelles pour les porcs a l'engrais. Rev. Agric. (Bruxelles), 36, 1713-1726.

Dierick, N.A., Vervaeke, I.J., Decuypere, J.A. and Hendrickx, H.K., 1986. Influence of the gut flora and some growth-promoting feed additives on nitrogen metabolism in pigs. II. Studies *in vivo*. Livest. Prod. Sci., 14: 177-193.

Drochner, W., 1984. Einfluß wechselnder Rohfaser- und Pekingehalte im Futter auf einige praecaecale und postileale Verdauungsvorgange beim waschsenchen Schwein. Forschritte Tierphysiol. Tierernaehr., 14, 1-125.

Dugan, M.E.R., Sauer, W.C., Dugan, J.M.Jr. and Caine, W.R., 1994. Determination of bacterial amino acid contribution to ileal digesta from pigs using ^{35}S and DAPA marker techniques. J. Anim. Feed Sci., 3, 149-159.

Dufva, G.S., Bartley, E.E., Arambel, M.J., Nagaraja, T.G., Dennis, S.M., Galitzer, S.J. and Dayton, A.D., 1982. Diaminopimelic acid content of feeds and rumen bacteria and its usefulness as a rumen bacterial marker. J. Dairy Sci., 65, 1754-1759.

Gill, J.L. and Magee, W.T., 1976. Balanced two-period changeover designs for several treatments. J. Anim. Sci., 42, 775-777.

Huber, W.G., 1982. Aminoglycosides, macrolides, lincosamides, polymyxins, chloramphenicol, and other antibacterial drugs. In: N.H. Booth and L.E. McDonald (Eds.), Veterinary Pharmacology and Therapeutics, Fifth Edition. The Iowa State University Press, Ames, IA, pp. 747-771.

Jones, B. N. and Gilligan, J.P., 1983. *o*-Phthaldialdehyde precolumn derivitization and reverse-phase high performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J. Chromatogr., 266, 471-482.

Laplace, J.P., Darcy-Vrillon, B., Duval-Iflah, Y. and Raibaud, P., 1985. Proteins in digesta of the pig: amino acid composition of endogenous, bacterial and faecal fractions. Reprod. Nutr. Develop., 25, 1083-1099.

Lien, K.A., Sauer, W.C. and Fenton, M., 1997. Recovery and composition of crude mucus in ileal digesta of pigs fed a protein-free diet. Z. Ernahrungswiss, 36, 182-190.

McAllister, J.S., Kurtz, H.J. and Short Jr., E.C., 1979. Changes in the intestinal flora of young pigs with postweaning diarrhea or edema disease. J. Anim. Sci., 49, 868-879.

National Research Council. 1988. Nutrient Requirements of Swine (9th rev. Ed.). National Academy Press, Washington, D.C.

Poppe, S., Meirer, H., Bennke, H.J. and Stuwe, E., 1983. Zur Protein und Aminosaeurenverdaulichkeit in verschiedenen Darmabschnitten beim Schwein. 3. Aminosaeurenzusammensetzung von Chymus- und Kotprotein. Arch. Tierernaehr., 33, 743-748.

Rooke, J.A., Fraser, H., Shanks, M. and Morgan, A., 1996. The potential for improving soya-bean meal in diets for weaned piglets by protease treatment: comparison with other protein sources. Proceedings of the 112th meeting of the British Society of Animal Science, Scarborough, UK. Anim. Sci. 62, 658.

Sauer, W.C. and Ozimek, L., 1986. Digestibility of amino acids in swine: results and their practical applications. A review. Livest. Prod. Sci. 15, 367-388.

Statistical Analysis System. 1990. SAS/STAT® User's Guide (Release 6.08). SAS Inst. Inc., Cary, NC.

Schulze, H., van Leeuwen, P., Verstegen, M.W.A., Huisman, J., Souffrant, W.B. and Ahrens, F., 1994. Effect of level of dietary neutral detergent fiber on ileal apparent digestibility and ileal nitrogen losses in pigs. J. Anim. Sci., 72, 2362-2368.

Seve, B. and Leterme, P., 1997. Amino acid fluxes in the pig. In: J.P. Laplace, C. Fevrier and A. Barbeau (Eds), Digestive Physiology In Pigs. EAAP Publication no. 88: 304-315.

Souffrant, W.B., 1991. Endogenous nitrogen losses during digestion in pigs. In: M.W.A. Verstegen, J. Huisman and L.A. den Hartog (Eds.), Digestive Physiology In Pigs: Endogenous Losses During Digestion In Pigs. Proc. 5th Int. Symp. On Digestive Physiology In Pigs. EAAP Publication no. 54, Pudoc, Wageningen. pp. 147-166.

Steel, R.G.D. and Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Publishing Co., New York.

Synge, R.L.M., 1953. Note on the occurrence of diaminopimelic acid in some intestinal micro-organisms from farm animals. J. Gen. Microbiol., 8, 407-409.

Tamminga, S., Schulze, H., van Bruchem, J. and Huisman, J., 1995. The nutritional significance of endogenous N-losses along the gastro-intestinal tract of farm animals. Arch. Anim. Nutr. 48, 9-22.

Wunsche, J., Herrmann, U., Meinl, M., Hennig, U., Kreienbring, F. and Zwierz, P., 1987. Einfluβ exogener Faktoren auf die prazakale Nahrstoff-und Aminosaurenresorption, ermittelt an Schweinen mit Ileo-Rektal-Anastomosen. 1. Mitt.: Einfluβ des Zerkleinerungsgrades von Getreide. Arch. Anim. Nutr., 37, 745-764.

Appendix 1

Recalculated endogenous recoveries (g/kg dry matter intake) and true ileal digestibilities (g/100 g) of amino acids for piglets fed the protease incubated soybean meal (PI-SBM) assuming that amino acids lost during guanidination would have been entirely digested.

	Endogenous Recoveries	SE[a]	True Digestibilities	SE[a]
Amino acids				
Indispensable				
Arginine	0.8	0.1	88.8	0.3
Histidine	1.7	0.1	110.6	1.2
Isoleucine	1.8	0.1	92.6	0.2
Leucine	1.7	0.1	85.0	0.6
Lysine[b]	1.1	0.1	85.8	0.4
Methionine	1.1	0.3	99.9	2.5
Phenylalanine	1.5	0.1	91.0	0.3
Threonine	2.8	0.2	95.3	0.2
Valine	2.2	0.1	92.2	0.2
Dispensable				
Alanine	2.8	0.1	97.5	0.2
Aspartate + Asparagine	1.5	0.1	82.4	0.8
Cysteine	1.5	0.2	100.8	1.5
Glutamate + Glutamine	1.3	0.2	81.0	1.3
Glycine	3.3	0.3	99.8	0.9
Serine	1.9	0.1	91.0	0.2
Tyrosine	1.6	0.1	102.2	0.8

[a] Standard error (n = 6).

[b] Lysine lost during guanidination was assumed to be converted to homoarginine at the same level as previously reported for PI-SBM (Caine et al., 1997c).

6 Exchange of Amino Acids and Peptides Across the Portal Vein-Drained Tissue of Pigs

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Exchange of Amino Acids and Peptides Across the Portal Vein-Drained Tissue of Pigs

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Abstract

An experiment was conducted to determine the exchange of amino acids across the portal vein-drained tissue into and out of the plasma, red blood cell and plasma peptide pools of the portal vein of growing pigs. The pigs, previously fitted with catheters in an occluded carotid artery and the portal and mesenteric veins, were fed a maize starch-based diet with wheat gluten as protein source. The feed was given daily at the level of 2 g/100 g body weight as a wet mash (1:2, wt/vol water) in two equal meals at 0800 and 1600 h. After 5-d adaptation to diet, the pigs received their 0800 h meal and, subsequently, were not fed again for 28 h. At 4 (fed) and 28 (unfed) h after receiving the meal, simultaneous 8 mL blood samples were taken from the portal vein and carotid artery of each pig. The flow of whole blood and plasma were estimated by the indicator-dilution method, using the infusion of *p*-aminohippuric acid. A second series of blood samples and flow measurements were collected 5 d later. Amino acid concentrations were determined in deproteinized whole blood and plasma and in acid hydrolysates of the plasma. Total exchange of amino acids was higher ($P = 0.02$) in the plasma (55.8 vs 1.9 mmol/h) and plasma peptide (123.2 vs -1.2 mmol/h) pools of fed compared to unfed pigs, respectively. Corresponding total exchange in the red blood cell pool (77.6 vs 6.0 mmol/h) was not statistically significant ($P = 0.29$) because of a large standard error from accumulated analytical variation. The exchange of most of the indispensable amino acids was higher ($P < 0.05$) in the plasma pool of fed than unfed pigs. Corresponding exchange of alanine, asparagine, serine and tyrosine were also higher ($P < 0.05$) in the fed pigs. The exchange of most of the amino acids in the plasma peptide pool of the portal vein blood of fed pigs was usually higher ($P < 0.05$) than that of unfed pigs. A substantial proportion of the amino acids in the plasma peptide pool were of dietary origin based on a similar amino acid profile to that of wheat gluten. In conclusion, amino acid exchange across the portal vein-drained tissue of pigs is a dynamic process that includes the plasma free amino acid and plasma peptide pools and probably red blood cells of portal vein blood.

Key Words: Pigs, Amino acids, Peptides, Red Blood Cells, Portal vein, Exchange

Introduction

Blood plasma is conventionally considered to be the main pool of exchange and transport of free amino acids. However, exchange of amino acids in red blood cells have been reported across the gastrointestinal and liver tissues of dogs (Elwyn et al., 1968), rats (Boullin et al., 1973; Galibois et al., 1991) and sheep (Heitmann and Bergman, 1980). Moreover, the exchange of amino acids across the gastrointestinal tissue was substantially higher in the form of plasma peptides than that of the plasma and red blood cell pools of the portal vein of fed and unfed calves (Koeln et al., 1993). The relationship between gastrointestinal exchange of amino acids and the different blood transport pools has not been reported in pigs. This is surprising considering that diet formulations for pigs are usually based on ileal amino acid digestibilities of dietary protein (Sauer and

Ozimek, 1986). The nutritive value of protein sources for pigs are also increasingly being evaluated by determining true ileal digestibilities of amino acids (Caine et al., 1997). This further emphasizes the importance of understanding what proportion of amino acids absorbed from the gastrointestinal tract are of dietary or endogenous origin. Indeed, Rerat (1990) has suggested that ileal amino acid digestibilities do not adequately represent the nutritive value of protein sources for pigs. Instead, he advocates the portal blood appearance of amino acids as a better indication of their availability for protein tissue synthesis. In this respect, Rerat et al. (1988) reported a higher and more rapid appearance of amino acids in the portal vein of pigs receiving a duodenal infusion of milk protein peptide hydrolysate than a free amino acid solution of the same composition. These authors did not report exchange of amino acids between the different blood transport pools but their results suggest that plasma peptides may be absorbed from the gastrointestinal tract of pigs.

The objective of this study was to determine the exchange of amino acids across the portal vein-drained tissue into the plasma, red blood cell and plasma peptide pools of the portal vein of fed and unfed pigs.

Experimental

Animals and diets. The experiment was carried out according to a repeated measures design (Steel and Torrie, 1980). Four cross-bred barrows [(Dutch Landrace × Yorkshire) × Finnish Landrace] with an average body weight of 54.2 ± 1.2 kg were fitted with indwelling catheters in the portal and mesenteric veins as described by van Leeuwen et al. (1995) and in an occluded carotid artery as described by Yen and Killefer (1987). The pigs were housed in individual metabolism crates (80 × 180 cm) in a temperature-controlled (23 ± 1°C) room at the TNO-ILOB Institute, Wageningen. The experimental diet was maize starch-based with wheat gluten as the only protein source. The diet contained 152.5 g crude protein/kg, as fed to the pigs. Soya oil was added so that the diet exceeded Centraal Veevoederbureau (CVB; 1995) standards for digestible energy. Vitamins and minerals were supplemented to the diet to meet or exceeded CVB (1995) standards. Formulation and nutrient concentrations of the diet are presented in **Table 1**.

The pigs were fed at a daily level of 2 g/100 g body weight in two equal meals at 0800 and 1600 h throughout the experiment. Feed was given as a wet mash (1:2, wt/vol water). No additional water was available to the pigs. Each experimental period lasted 7 d. From d 1 to 5, the pigs were fed the experimental diet. At 0600 h on d 6 the

mesenteric vein catheter of each pig was connected to a perfusion pump (B. Braun Melsungen AG, FRG) for priming and subsequent constant infusion of 0.9 g NaCl/L solution containing 10 g/L *p*-aminohippuric acid (PAH) as blood flow marker. A swivel apparatus as described by van Kleef (1993) allowed the pigs to move freely during the

Table 1.
Formulation and nutrient concentration of the experimental diet as fed to the pigs.

Ingredient, g/kg		Nutrient, g/kg[a]	
Maize starch	525	Dry matter	888.1
Wheat gluten	178	Crude Protein[b]	152.5
Dextrose	150	Amino acids	
Cellulose	50	Indispensable	
$CaHPO_4 \cdot H_2O$	25	Arg	7.1
Soya oil	20	His	4.5
$KHCO_3$	20	Iso	6.6
$CaCO_3$	10	Leu	13.5
Vitamin/mineral premix[c]	10	Lys	3.8
NaCl	5	Met	2.5
$NaHCO_3$	5	Phe	10.0
$MgSO_4 \cdot 7H_2O$	2	Thr	3.6
		Val	8.4
		Dispensable	
		Ala	4.2
		Asp + Asn	5.9
		Glu + Gln	63.0
		Gly	11.4
		Ser	7.5
		Tyr	3.2

[a] Nutrient concentration as analyzed.

[b] Crude protein; nitrogen × 6.25.

[c] Provided the following (mg/kg of diet): choline, 1000.0; ascorbate, 50.0; α-tocopheryl acetate, 40.0; niacin, 30.0; d-pantothenate, 12.0; riboflavin, 5.0; retinyl palmitate, 4.95; menadione dimethyl-pyrimidinol bisulphite, 3.0; pyridoxal-phosphate, 3.0; thiamin, 2.0; folacin, 1.0; d-biotin, 0.1; cholecalciferol, 0.045; cyanocobalamin, 0.04; $ZnSO2_4 \cdot H_2O$, 300.0; MnO_2, 15.0; $FeSO_4 \cdot 7H_2O$, 400.0; $CoSO_4 \cdot 5H_2O$, 2.5; KI, 0.5; $CuSO_4 \cdot 5H_2O$, 35.0; $Na_2SeO_3 \cdot 5H_2O$, 0.2; tylosin, 40.0.

infusion of the PAH solution. Four h after receiving the 0800 h morning meal on d 6, 8 mL blood samples were simultaneously withdrawn from the portal vein and carotid artery catheters of each pig. Infusion of the PAH was then discontinued. The pigs did not receive their next two meals; 1600 h on d 6 and 0800 h on d 7. Infusion of the PAH solution was repeated on d 7, as previously described, until 1200 h (28 h after receiving the morning meal on the previous day) when simultaneous blood samples were again withdrawn from the pigs. After collection of the blood samples the pigs were immediately given a meal. Blood samples collected at 4 and 28 h after receiving the 0800 h morning meal represented fed and unfed status of the pigs, respectively. The pigs were fed for an additional 5 d and then a second set of blood samples were collected at 4 and 28 h after receiving a morning meal, as previously described.

The experimental proposal and surgical procedures were approved by the TNO Committee for Animal Welfare.

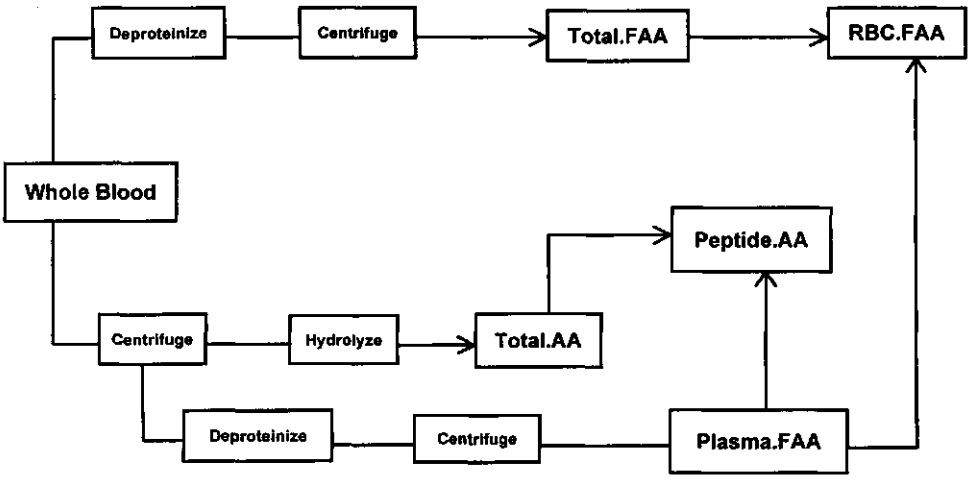

Figure 1.
A schematic diagram of the processing of whole blood samples to determine the concentration of plasma free amino acids (Plasma.FAA) and red blood cell free amino acids (RBC.FAA = Total.FAA - Plasma.FAA) and concentration of amino acids of the plasma peptides (Peptide.AA = Total.AA - Plasma.FAA).

Chemical analyses. A schematic diagram of the processing of blood samples representing the plasma and red blood cell free amino acid pools and the plasma peptide pool is presented in **Figure 1**. Samples of blood, stored on ice immediately following withdrawal from the pigs, were processed within 30 min after their collection. Packed cell volume was determined on the blood samples using a microhematocrit. A 2 mL aliquot, representing whole blood, was deproteinized by vortexing (Janke and Kunkel IKA-WERK, Model# VF-1, Staufen im Breisgan, D) in 18 × 150-mm test tubes with the slow addition of an equal volume of cold 1.5 N Trichloroacetic acid (chilled in ice water). The test tubes were centrifuged at 4 °C and 3000 × *g* for 10 min and the supernatant transferred to a second test tube and frozen at -40 °C. The remaining portion of the blood samples were centrifuged at 4 °C and 3000 × *g* for 10 min to remove the red blood cells and the plasma subdivided into three aliquots. Two 1 mL aliquots were frozen at -40 °C until later analysis for PAH and plasma peptide amino acid concentrations, respectively. A 2 mL aliquot, representing plasma, was deproteinized as described for whole blood and frozen at -40 °C. Subsequently, the whole blood and plasma aliquots were freeze-dried and stored at -20 °C until amino acid analysis.

The dry matter content of the diet was determined according to Association of Official Analytical Chemists (1990). The crude protein (nitrogen × 6.25) content in the diet was measured with an Automated Nitrogen Analyzer (FP-428 Nitrogen Determinator, Leco® Corporation, St. Joseph, MI). Analysis of amino acid concentrations in blood samples and the diet were performed by a fluorometric method involving pre-column derivatization with *o*-phthaldialdehyde and analysis by High Performance Liquid Chromatography (HPLC) according to Sedgwick et al. (1991). A Varian 5000 HPLC system with a Varian 9090 autosampler and a Varian Fluorichrom detector (excitation 340 nm, emission 450 nm; Varian Canada Inc., Mississauga, ON) was used for the analysis. Whole blood and plasma samples were reconstituted in 2 mL of deionized water prior to analysis. The concentration of amino acids in plasma peptides and the diet were determined by hydrolyzing 1 mL of plasma or 100 mg of the diet in 13 × 100-mm screw-capped culture tubes containing 3 mL of 6 mol/L HCl. The tubes were purged with nitrogen before sealing the screw cap and then incubated in an oven at 100°C for 24 h. Concentrations of amino acids were determined in duplicate aliquots of the deproteinized whole blood and plasma samples or hydrolysates, injected on a Supelcosil 3 micron LC-18 reverse phase column (4.6 × 150-mm; Supelco, Sigma-Aldrich Canada Ltd., Mississauga, ON) equipped with a Supelco LC-18 reverse phase 20 to 40 micron guard column (4.6 × 50-mm). Peaks for amino acids were recorded and integrated with the EZchrom™ Chromatography Data System (version 2.12, Shimadzu

Scientific Instruments Inc., Columbia, MD). Difference in the amino acid concentrations of deproteinized whole blood and plasma hydrolysates from that of deproteinized plasma represented the values for red blood cells and plasma peptides, respectively. Concentration of amino acids were reported on a whole blood basis with values for the plasma and plasma peptide pools corrected for a 20% content of plasma in the packed cell volume according to Koeln et al. (1993).

Calculation of portal vein blood flow. The concentration of PAH in plasma samples was analyzed according to the procedure described by Katz and Bergman (1969). The flow of whole blood and plasma in the portal vein were calculated by the indicator dilution technique, using PAH as blood flow marker and the equations of Yen and Killefer (1987).

Statistical analysis. The concentration and exchange of amino acids and portal vein blood flows were subjected to analysis of variance using a split-plot model (General Linear Model Procedure; Statistical Analysis System Institute Inc., 1990) with samples collected within pigs taken as repeated measurements. The statistical model was, as follows:

$$Y_{ijk} = \mu + T_i + A_k(T_i) + P_j + (T_i \times P_j) + \epsilon_{ijk}$$

where; Y_{ijk}, dependent variable; μ, overall mean; T, time after feeding (i = 2); P, experimental period (j = 2); A, animal (k = 4) and ϵ_{ijk}, residual error. The effect of time after feeding (T) was tested against animal within time after feeding [A(T)]. The effect of period (P) and the time after feeding by period interaction (T × P) were tested against the residual error (ϵ_{ijk}). Means of whole blood and plasma flow in the portal vein and the concentration and exchange of amino acids in fed and unfed pigs were compared using the Students' *t*-test according to Steel and Torrie (1980). The Students' *t*-test was also used to determine if the exchange of amino acids from the portal vein-drained tissue into the different transport pools of portal vein blood differed from zero. Means were considered to be different when the *P* value was less than 0.05. There was a trend for means to be different when the *P* value was less than 0.1.

Results and Discussion

The objective of the experiment was to determine the role of the different blood transport pools during digestion and absorption of wheat gluten as protein source in fed versus unfed pigs. Wheat gluten was chosen as the protein source because the amino acids are almost completely digested and absorbed (Sarwar et al., 1989). The amino

acid and peptide transport systems across the intestinal mucosa of mammals have not been fully characterized or well understood (Webb, 1990; Webb and Bergman, 1991). In this context, synthetic amino acids were not supplemented in the diet so that wheat gluten was the only protein source. With the exception of lysine and threonine amino acid concentrations in the diet met or exceeded requirements for optimum growth in pigs according to the CVB (1995). The pigs were fed at a level slightly above maintenance to minimize their weight gain while maintaining a positive nutritional status. The average daily gain of the pigs was 87.5 ± 12.7 g/d during the experiment.

There was usually no effect ($P > 0.1$) of period or time after feeding by period interaction for whole blood and plasma flow rates or arterial and portal vein blood concentrations and exchange of amino acids in the pigs.

Table 2.
Flow rates of whole blood and plasma in the portal vein of fed and unfed pigs.

Time after feeding, h	Whole Blood Flow, L/min	Plasma Flow, L/min
4.0 (Fed)	2.67[b]	2.20[b]
28.0 (Unfed)	1.77[c]	1.43[c]
SEM[a]	0.34	0.28
Overall Mean	2.22	1.82

[a] Standard error of the mean ($n = 8$).
[b,c] Means of fed and unfed pigs were different ($P < 0.05$).

Portal vein blood flow. The flow of whole blood and plasma in the portal vein of the fed and unfed pigs are presented in **Table 2**. The flow rates of the fed (4 h after feeding) pigs were higher ($P < 0.05$) than that of unfed (28 h after feeding) pigs. Whole blood flow rates were similar to values reported for fed (Rerat et al., 1988) and unfed (Yen and Killefer, 1987) pigs of similar weight. The average packed cell volume in arterial (29.0% ± 0.7) and portal vein (29.3% ± 0.8) blood was similar ($P > 0.1$).

Concentration of amino acids in the pools of arterial and portal vein blood. The concentration of amino acids in the plasma, red blood cell and plasma peptide pools of arterial and portal vein blood are presented in **Table 3** and **4**, respectively. In both the fed and unfed pigs 40 to 60 µmol/100 µmol of total amino acids were in the plasma pool of arterial and portal vein blood with the remaining 40 to 60 µmol/100 µmol more or less equally distributed between red blood cells and plasma peptides. It should be pointed

Table 3.
Concentration (μmol/L) of amino acids in the plasma, red blood cell and plasma peptide pools of arterial blood of fed and unfed pigs.

	Plasma				Red Blood Cells				Plasma Peptides			
	Fed	Unfed	SE[a]	*P*[b]	Fed	Unfed	SE[a]	*P*[b]	Fed	Unfed	SE[a]	*P*[b]
TAA[c]	2186	1536	123	0.01	851	575	66	0.03	542	737	207	0.53
IAA[c]	811	606	69	0.08	131	124	25	0.85	130	218	77	0.45
Arg	83.8	53.5	6.2	0.01	4.9	-1.4	4.5	0.36	5.2	13.2	7.1	0.45
His	46.5	28.4	5.4	0.05	14.7	4.3	6.3	0.29	11.4	14.4	4.8	0.67
Iso	96.6	72.0	6.9	0.05	2.2	6.9	1.9	0.12	12.1	20.9	8.1	0.47
Leu	133.5	82.6	12.6	0.03	16.2	14.4	2.5	0.62	40.0	48.4	13.4	0.68
Lys	32.8	78.1	10.3	0.02	54.8	50.4	7.6	0.70	28.4	45.0	12.5	0.38
Met	0.0	0.0	0.0	1.00	0.0	0.0	0.0	1.00				
Phe	88.1	39.8	7.5	0.01	4.0	3.7	0.9	0.84	20.6	24.7	7.7	0.72
Thr	155.3	115.0	18.2	0.17	17.8	25.0	6.8	0.49	-19.5	12.8	14.7	0.17
Trp	1.9	0.7	0.4	0.06	1.6	0.9	0.7	0.53				
Val	172.9	136.2	16.5	0.17	14.4	19.5	4.4	0.44	23.8	39.1	12.9	0.43
DAA[c]	1374	929	68	0.01	720	452	49	0.01	413	519	132	0.59
Ala	279.0	157.9	19.6	0.01	39.8	19.9	3.5	0.01	64.9	61.1	20.7	0.90
Asn	49.0	32.7	3.6	0.02	-2.1	0.8	1.5	0.22				
Asp	8.6	4.1	0.4	0.01	21.0	16.1	2.7	0.25	54.5	71.9	15.0	0.44
Cit	19.0	3.6	2.1	0.01	9.9	2.1	1.7	0.02				
Gln	96.4	54.4	7.8	0.01	28.6	14.8	7.9	0.26				
Glu	105.0	47.5	6.9	0.01	37.4	39.6	7.1	0.83	296.7	189.2	42.1	0.12
Gly	474.5	427.1	35.1	0.38	223.0	90.6	20.3	0.01	17.7	158.5	49.5	0.09
Orn	77.9	24.9	3.3	0.01	57.2	30.0	5.0	0.01				
Ser	122.8	91.9	8.0	0.03	23.3	13.8	2.8	0.05	16.7	47.6	15.7	0.21
Tau	65.2	49.8	5.9	0.12	269.6	209.8	24.5	0.13				
Tyr	77.4	35.6	5.9	0.01	12.5	14.2	1.9	0.54	-26.3	-13.9	8.3	0.34

[a] Standard error of the mean (n = 8).
[b] Probability that the means of fed and unfed pigs are different.
[c] Total amino acids, TAA; Indispensable amino acids, IAA; dispensable amino acids, DAA.

Table 4.
Concentration (μmol/L) of amino acids in the plasma, red blood cell and plasma peptide pools of portal vein blood of fed and unfed pigs.

	Plasma				Red Blood Cells				Plasma Peptides			
	Fed	Unfed	SE[a]	*P*[b]	Fed	Unfed	SE[a]	*P*[b]	Fed	Unfed	SE[a]	*P*[b]
TAA[c]	2582	1514	72	0.01	1677	650	482	0.18	1066	641	230	0.24
IAA[c]	967	588	56	0.01	367	142	143	0.31	289	182	94	0.45
Arg	103.9	51.8	4.6	0.01	21.4	2.4	10.8	0.26	18.6	10.0	7.9	0.47
His	66.4	27.5	2.8	0.01	14.2	7.8	5.1	0.40	24.9	13.3	13.5	0.57
Iso	112.5	69.2	8.1	0.01	34.8	9.2	20.2	0.41	23.8	19.9	8.6	0.76
Leu	168.7	79.3	8.8	0.01	69.0	21.1	28.1	0.27	61.8	43.2	12.3	0.33
Lys	43.9	76.7	10.3	0.07	77.1	52.4	18.9	0.39	54.8	32.0	14.7	0.32
Met	0.4	0.0	0.3	0.36	0.4	0.0	0.3	0.36				
Phe	111.0	38.2	7.1	0.01	29.0	6.2	16.7	0.37	30.2	22.6	8.0	0.52
Thr	166.7	113.1	16.1	0.06	55.8	19.1	22.1	0.28	21.5	5.4	18.3	0.56
Trp	2.2	0.3	0.3	0.01	1.4	1.2	0.7	0.90				
Val	191.0	132.4	14.8	0.03	63.5	22.1	30.7	0.38	52.9	34.6	14.5	0.41
DAA[c]	1616	926	38	0.01	1310	508	340	0.15	777	459	142	0.16
Ala	360.8	160.2	19.1	0.01	145.4	25.9	65.9	0.25	42.7	49.1	23.1	0.85
Asn	61.0	33.9	3.9	0.01	15.4	1.2	10.9	0.39				
Asp	11.8	4.5	1.8	0.03	22.9	15.4	4.5	0.29	102.8	71.8	14.4	0.18
Cit	22.8	3.8	2.4	0.01	13.0	6.1	2.8	0.14				
Gln	76.0	42.3	15.4	0.17	117.8	19.6	44.6	0.17				
Glu	104.0	43.6	6.6	0.01	72.7	42.5	17.6	0.27	476.5	177.0	24.3	0.01
Gly	564.8	434.8	20.3	0.01	366.5	109.9	89.4	0.09	110.5	125.8	51.1	0.84
Orn	85.9	24.6	3.3	0.01	93.1	28.8	19.9	0.06				
Ser	168.8	89.2	6.3	0.01	87.1	21.4	37.0	0.25	59.7	50.6	19.2	0.75
Tau	73.5	54.6	5.4	0.05	335.4	221.8	47.6	0.14				
Tyr	86.3	34.7	5.5	0.01	40.8	15.5	14.3	0.26	-34.1	-10.2	6.7	0.05

[a] Standard error of the mean (n = 8).
[b] Probability that the means of fed and unfed pigs are different.
[c] Total amino acids, TAA; Indispensable amino acids, IAA; dispensable amino acids, DAA.

out that amino acid concentrations of red blood cells and plasma peptides were calculated as the difference between the amino acids concentrations of deproteinized whole blood and acid hydrolysates of plasma and those of plasma (Figure 1). In this context, inaccuracies in the determination of estimates for both the red blood cell and plasma peptide pools accumulated resulting in large standard errors that often prevented probable differences from being statistically significant. Nevertheless, the total concentration of amino acids in the plasma and red blood cell pools of arterial blood of fed pigs was higher ($P < 0.05$) than that of unfed pigs. Corresponding plasma peptide pools were similar ($P = 0.53$). In portal vein blood only the plasma free amino acid pool was different ($P = 0.01$) between fed and unfed pigs. Although, the means of the red blood cell and plasma peptide pools of fed pigs were numerically higher than that of unfed pigs but not statistically different ($P > 0.1$) for the reason previously discussed. The distribution of amino acids in the plasma peptide pool of the portal vein did not change much between fed and unfed pigs. Whereas, distribution of total, indispensable and dispensable amino acids increased 36.0, 67.7 and 25.7 μmol/100 μmol, respectively, in the plasma peptide pool of arterial blood of unfed compared to fed pigs. This suggests that the peripheral tissues of unfed pigs take over the role of the gastrointestinal tract for providing circulating plasma peptides to the liver. It could also be speculated that the substantial increase in the proportion of indispensable amino acids in the form of peptide in the arterial blood of unfed pigs represents a mobilization of tissues such as skeletal muscle to support the synthesis of essential proteins like enzymes in the liver. With respect to individual amino acids, the concentrations of methionine and tryptophan in the samples were usually low (below analytical detection limits) and, therefore, considered to be zero. The plasma concentration of most of the amino acids were higher ($P < 0.05$) in the fed than unfed pigs in both arterial and portal vein blood. The exception was lysine concentration which was lower ($P = 0.02$) in arterial plasma and tended ($P = 0.07$) to be lower in portal vein plasma of the fed pigs. The difference in alanine concentration between fed and unfed pigs was the highest of the amino acids at 121.1 and 200.6 μmol/L for arterial and portal vein plasma, respectively. These increases in alanine concentration emphasize the metabolic activity of the gastrointestinal tract of the fed pigs. Alanine is the predominant amino acid released by the jejunum of growing pigs (Wu et al., 1994).

The arterial red blood cell concentration of the dispensable amino acids, alanine, citrulline, glycine, ornithine and serine, were higher ($P < 0.05$) in the fed than unfed pigs. None of the portal vein red blood cell free amino acid concentrations were different ($P > 0.05$) in fed than unfed pigs, although, there was a trend for an increase in glycine

Table 5.

Exchange (mmol/h) of total and indispensable amino acids across the portal vein-drained tissue into and out of the plasma, red blood cell and plasma peptide pools of the portal vein blood of fed and unfed pigs.

	Plasma						Red Blood Cells						Plasma Peptide					
	Fed[a]	*P*[b]	Unfed	*P*[b]	SE[c]	*P*[d]	Fed	*P*[b]	Unfed	*P*[b]	SE[c]	*P*[d]	Fed	*P*[b]	Unfed	*P*[b]	SE[c]	*P*[d]
TAA[e]	55.8	0.01	1.9	0.88	11.7	0.02	77.6	0.13	6.0	0.89	43.6	0.29	123.2	0.01	-1.2	0.96	29.6	0.02
IAA[e]	22.6	0.01	-0.6	0.92	5.4	0.02	21.1	0.17	1.5	0.92	13.3	0.34	37.2	0.01	-0.7	0.96	10.9	0.05
Arg	2.8	0.01	0.1	0.94	0.8	0.05	1.6	0.19	0.2	0.90	1.1	0.38	3.3	0.02	-0.2	0.88	1.1	0.06
His	2.7	0.03	0.0	0.98	0.9	0.08	-0.1	0.95	0.4	0.73	1.2	0.77	4.2	0.09	0.2	0.91	2.0	0.22
Iso	2.5	0.01	-0.2	0.80	0.6	0.02	2.7	0.20	0.2	0.92	1.9	0.38	2.9	0.02	0.1	0.98	1.0	0.09
Leu	5.2	0.01	0.0	0.98	1.0	0.01	5.0	0.07	0.6	0.82	2.3	0.22	5.7	0.01	-0.1	0.95	1.4	0.03
Lys	1.7	0.01	0.0	0.98	0.3	0.01	2.2	0.17	0.2	0.89	1.4	0.35	4.8	0.07	-0.7	0.76	2.2	0.12
Met	0.1	0.21	0.0	1.00	0.1	0.36	0.1	0.21	0.0	1.00	0.0	0.36						
Phe	3.3	0.01	-0.1	0.92	0.5	0.01	2.1	0.23	0.2	0.89	1.5	0.43	3.0	0.02	0.0	0.98	0.9	0.06
Thr	1.6	0.19	-0.1	0.90	1.1	0.29	3.4	0.19	-0.6	0.79	2.3	0.26	7.9	0.01	0.0	0.99	2.2	0.05
Trp	0.0	0.73	0.0	0.79	0.1	0.67	0.0	0.91	0.1	0.79	0.2	0.91						
Val	2.7	0.02	-0.2	0.82	0.9	0.06	4.1	0.21	0.3	0.92	2.9	0.39	6.0	0.01	-0.1	0.97	1.5	0.03

[a] Negative means suggest that more of the total, indispensable or individual amino acids entered than left the portal vein-drained tissue.

[b] Probability that the mean does not differ from zero.

[c] Standard error of the mean (n = 8).

[d] Probability that the means of fed and unfed pigs are different.

[e] Total amino acids, TAA; Indispensable amino acids, IAA.

($P = 0.09$) and ornithine ($P = 0.06$). However, a higher ($P = 0.03$) concentration of total amino acids in the arterial red blood cells of fed compared to unfed pigs suggests that this blood pool has a role in the transport of free amino acids that has also been reported for other animals. For instance, transport of amino acids in red blood cells have been reported in cattle (Hanigan et al., 1991; Koeln et al., 1993), dogs (Elwyn, 1966; Elwyn et al., 1968), sheep (Young and Ellory, 1977; Heitmann and Bergman, 1980) and humans (Felig et al., 1973). Red blood cells may also have an ancillary function in the renal-intestinal transport flux of urea cycle intermediates based on the concentrations of citrulline ($P = 0.02$) and ornithine ($P = 0.01$) in the arterial red blood cells of the fed pigs. Arginine is not an indispensable amino acid in older pigs because of it's *de novo* synthesis in the kidneys using citrulline as a precursor (Wu et al., 1994). Hanigan et al. (1991) classified the red blood cell transport of citrulline and ornithine as being counter current and equal with plasma, respectively, based on arterial-venous differences in dairy cows. The red blood cells of pigs may have a similar role.

The concentration of amino acids in the plasma peptide pools of arterial and portal vein blood were usually similar ($P > 0.05$) between fed and unfed pigs. The notable exception was glutamate concentration which was 2.7 times higher ($P = 0.01$) in the portal vein plasma peptide pool of fed compared to unfed pigs. This difference suggests that a substantial proportion of glutamate, which has a high concentration in wheat gluten, was absorbed by the intestinal mucosa and then transported in the form of peptide.

Exchange of amino acids across the portal vein-drained tissues. The exchange of total and indispensable amino acids across the portal vein-drained tissue into and out of the plasma, red blood cell and plasma peptide pools of the portal vein blood of fed and unfed pigs is presented in **Table 5**. The total exchange of amino acids in the plasma (55.8 mmol/h) and plasma peptide (123.2 mmol/h) pools of fed pigs differed ($P = 0.01$) from zero and were higher ($P = 0.02$) than for unfed pigs. For reasons previously discussed, total exchange of amino acids (77.6 mmol/h) in the red blood cells of fed pigs did not reach a statistically significant ($P = 0.13$) difference from zero and were not different ($P = 0.29$) than that of the red blood cells (-1.2 mmol/h) of unfed pigs. Total exchange of amino acids in all of the blood pools of unfed pigs did not differ ($P > 0.1$) from zero. The total exchange of indispensable amino acids was higher in the plasma ($P = 0.02$) and plasma peptide ($P = 0.05$) pools of fed compared to unfed pigs. Although, there was usually no difference ($P > 0.1$) between fed and unfed pigs for indispensable amino acids with a low concentration in the diet.

Table 6.
Exchange (mmol/h) of dispensable amino acids across the portal vein-drained tissue into and out of the plasma, red blood cell and plasma peptide pools of the portal vein blood of fed and unfed pigs.

	Plasma						Red Blood Cells						Plasma Peptide					
	Fed[a]	*P*[b]	Unfed	*P*[b]	SE[c]	*P*[d]	Fed	*P*[b]	Unfed	*P*[b]	SE[c]	*P*[d]	Fed	*P*[b]	Unfed	*P*[b]	SE[c]	*P*[d]
DAA[e]	33.2	0.01	2.4	0.74	6.9	0.02	56.5	0.12	4.6	0.89	31.2	0.28	86.0	0.01	-0.5	0.96	19.0	0.01
Ala	11.6	0.01	0.6	0.58	1.0	0.01	9.3	0.21	0.6	0.94	6.7	0.39	0.8	0.84	-0.7	0.84	3.6	0.78
Asn	1.8	0.01	0.2	0.56	0.4	0.03	1.4	0.25	0.0	0.99	1.1	0.40						
Asp	0.6	0.10	0.1	0.80	0.3	0.28	-0.5	0.21	-0.1	0.83	0.3	0.43	10.3	0.01	0.5	0.86	2.9	0.06
Cit	0.4	0.08	0.1	0.82	0.2	0.24	0.2	0.47	0.4	0.27	0.3	0.76						
Gln	-1.7	0.59	-1.2	0.69	2.9	0.92	6.3	0.10	0.5	0.89	3.2	0.25						
Glu	-0.5	0.44	-0.4	0.50	0.6	0.94	3.8	0.05	0.5	0.77	1.5	0.17	38.0	0.01	0.0	0.99	6.9	0.01
Gly	11.2	0.01	2.4	0.46	3.0	0.08	16.4	0.14	0.9	0.93	9.7	0.30	26.0	0.02	-1.9	0.82	7.8	0.05
Orn	0.8	0.04	0.0	0.96	0.3	0.11	4.0	0.06	-0.1	0.98	1.7	0.15						
Ser	6.5	0.01	0.1	0.94	1.1	0.01	5.9	0.14	0.6	0.87	3.4	0.32	11.3	0.01	1.1	0.60	1.9	0.01
Tau	1.0	0.14	0.7	0.31	0.6	0.68	7.4	0.11	1.2	0.77	4.0	0.31						
Tyr	1.4	0.03	0.0	0.98	0.5	0.08	2.5	0.12	0.1	0.95	1.4	0.27	-0.4	0.67	0.5	0.61	1.0	0.51

[a] Negative means suggest that more of the dispensable or individual amino acids entered than left the portal vein-drained tissue.
[b] Probability that the mean does not differ from zero.
[c] Standard error of the mean (n = 8).
[d] Probability that the means of fed and unfed pigs are different.
[e] Dispensable amino acids, DAA.

The exchange of dispensable amino acids across the portal vein-drained tissue into and out of the plasma, red blood cell and plasma peptide pools of the portal vein blood of fed and unfed pigs is presented in **Table 6**. The exchange of dispensable amino acids was different ($P = 0.01$) from zero in the plasma and plasma peptide pools of fed pigs. Alanine, glycine and serine accounted for 35, 34 and 20 mmol/100 mmol, respectively, of the total exchange of dispensable free amino acids in the plasma pool of the fed pigs. The metabolic fate of a large proportion of these amino acids was probably gluconeogenesis in the liver. Moreover, the exchange of glutamate ($P = 0.05$) and glutamine ($P = 0.1$) in red blood cells of the fed pigs suggests that this blood pool has a counter current role to plasma in transporting amino acids destine for deamination to α-ketoglutarate and subsequent glucose synthesis in the liver. Hanigan et al. (1991) discussed the counter current role of red blood cells for transporting amino acids in the opposite direction of plasma in dairy cows.

Exchange of indispensable and dispensable amino acids into the plasma peptide pool of the portal vein blood of fed pigs was about one and a half and three times greater than for the plasma free amino acids, respectively. This indicates that a substantial proportion of the amino acids transported as plasma peptides were probably absorbed in a similar form from the lumen of the gastrointestinal tract. Growing pigs receiving an intraduodenal infusion of an enzymatic hydrolysate of milk protein, comprised primarily of small peptides, had greater and more rapid absorption of amino acids than pigs receiving an equivalent mixture of free amino acids (Rerat et al., 1988). The importance of peptide absorption from the portal vein-drained tissue was also reported in rats (Galibois et al. 1991) and Holstein steers (Koeln et al. 1993). What proportion of these plasma peptide amino acids came from the diet or are of endogenous origin is unknown.

Dietary and endogenous origin of amino acids in the blood pools. Wheat gluten was used as protein source in the present study because the amino acids are assumed to be almost 100% digestible (Sarwar et al. 1989). Furthermore, wheat gluten has a very high content of glutamate. The amino acids recovered at the terminal ileum of pigs fed wheat gluten as protein source can be considered to be of endogenous origin. The composition of protein recovered at the terminal ileum of pigs fed wheat gluten has a high concentration of amino acids that typically also have a high concentration in endogenous secretions such as pancreatic and mucus secretions and bile (P. van Leeuwen, personal communication). In this context, the amino acids in the blood transport pools were assumed to have originated from the diet or from endogenous sources that include salivary, pancreatic, bile and intestinal secretions, mucus and

epithelial cells (Souffrant, 1991). Therefore, the origin of the amino acids exchanged into the plasma peptide pool of the portal vein blood of fed pigs should have a similar profile to those of wheat gluten and endogenous secretions. Metabolic activity of the gastrointestinal tissue would have, for the most part, altered the composition of free amino acids in the plasma and red blood cell pools and, in this respect, obfuscate any similarity to the composition of dietary protein or endogenous secretions. With the exception of amino acids that have a high concentration in endogenous mucus such as threonine and serine (e.g. Lien et al., 1997) or in pancreatic (e.g. Gabert et al., 1996) secretions such as glutamate, aspartate and the branched-chain amino acids, the proportion of amino acids in the diet and plasma peptides of the fed pigs more or less followed a similar pattern. Glutamate and glycine accounted for 30.8 and 21.1 mmol/ 100 mmol, respectively, of the total exchange of amino acids in the plasma peptide pool of the portal vein blood of fed pigs. Glutamate also had the highest concentration in the diet at 40.6 g/100 g of the amino acids reported. The corresponding concentration of glycine in the diet was 7.3 g/100 g. This suggests that a substantial proportion of the plasma peptides in the portal vein blood of the fed pigs were of dietary origin. The high exchange of glycine and to a lessor extent taurine into the plasma and red blood cell free amino acid pools of the portal vein blood of the fed pigs would have, in part, originated from the reabsorption of bile acids secreted into gastrointestinal lumen.

The secretion of endogenous nitrogen into the gastrointestinal tract of pigs ranges from 38.0 to 60.1 g/100 g of nitrogen intake (Auclair, 1986; as cited by Souffrant, 1991). The proportion of amino acids from endogenous and dietary origin in the plasma peptide pool of the portal vein blood of fed pigs would be in the same order based on the amino acid profiles of wheat gluten and mucus (Lien et al., 1997) and pancreatic (Gabert et al., 1996) secretions. Nevertheless, there is still skepticism about the role of peptides in the absorption and exchange of amino acids from portal vein-drained tissue based on the substantial activity of proteases and peptidases in the intestinal lumen and cytosol of enterocytes (Webb, 1990). In the present study, glutamate must have been absorbed and transported in the form of plasma peptide based on the high concentration of this amino acids in wheat gluten and because of it's uptake by the gastrointestinal tract (Heitmann and Bergman, 1980; Koeln et al., 1993). As previously mentioned, red blood cells also have a counter current role in the transport of this amino acid. On the other hand, the proportion of alanine in the plasma peptide pool was low with a corresponding high contribution to the plasma free amino acid pool. The highest proportion of lysine and arginine were in the plasma peptide pool. These amino acids are the first to be released from dietary protein because trypsin hydrolyzes the peptide

bond next to basic amino acids. As such, they have a high apparent digestibility (Low 1980). Similarly, a large proportion of the branched-chain amino acids were also in the peptide pool and this may be due to their early hydrolysis from dietary protein by chymotrypsin. Their apparent digestibility is also high relative to other amino acids (Low, 1980). In this context, amino acid digestibilities of dietary protein may partly depend on the proportion of the amino acids absorbed in the form of plasma peptide. Based on the results of the present study the potential for manipulating the proportion of dietary amino acids that are absorbed from the gastrointestinal tract of pigs as plasma peptides warrants further study.

In conclusion, the exchange of amino acids into the portal vein blood of fed pigs was quantitatively higher in the form of plasma peptides than plasma free amino acids. Red blood cells probably also have a role in the exchange of amino acids. A large proportion of dietary amino acids were exchanged into the portal vein blood of fed pigs in the form of plasma peptides. Difference in the digestibility of dietary protein may, in part, be dependent on the proportion of amino acids absorbed as peptides.

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Literature Cited

Association of Official Analytical Chemists. 1990. Official Methods of Analysis. (15th Ed.). AOAC. Arlington, VA.

Boullin, D.J., Crampton, R.F., Heading, C.E. and Pelling, D., 1973. Intestinal absorption of dipeptides containing glycine, phenylalanine, proline, β-alanine, or histidine in the rat. Clin. Sci. Mol. Med., 45, 849-858.

Caine, W.R., Tamminga, S., Verstegen, M.W.A., Sauer, W.C. and Schulze, H., 1997. Endogenous recoveries and true ileal digestibilities of amino acids in newly weaned piglets fed diets with protease-treated soybean meal. J. Anim. Sci. In press.

Centraal Veevoederbureau (CVB). 1995. [Tables for Feedstuffs. Data on the chemical composition, digestibility and feeding value of feed ingredients.] Centraal Veevoederbureau, Lelystad, The Netherlands.

Elwyn, D.H., 1966. Distribution of amino acids between plasma and red blood cells in the dog. Fed. Proc., 25, 854-861.

Elwyn, D.H., Parikh, H.C. and Shoemaker, W.C., 1968. Amino acid movements between gut, liver and periphery in unanesthetized dogs. Am. J. Physiol., 215, 1260-1275.

Felig, P., Wahren, J. and Raf, L., 1973. Evidence of inter-organ amino acid transport by blood cells in humans. Proc. Natl. Acad. Sci. USA, 70, 1775-1779.

Gabert, V.M., Sauer, W.C., Li, S. and Fan, M.Z., 1996. Exocrine pancreatic secretions in young pigs fed diets containing fababeans (*Vicia faba*) and peas (*Pisum sativum*): concentrations and flows of total, protein-bound and free amino acids. J. Sci. Food Agric. 70, 256-262.

Galibois, I., Pitre, F., Parent, G. and Savoie, L., 1991. Analysis of bound amino acids in the plasma of fed rats: a new preparation procedure. J. Nutr. Biochem., 2, 25-30.

Hanigan, M.D., Calvert, C.C., DePeters, E.J., Reis, B.L. and Baldwin, R.L., 1991. Whole blood and plasma amino acid uptakes by lactating bovine mammary glands. J. Dairy Sci., 74, 2484-2490.

Heitmann, R.N. and Bergman, E.N., 1980. Transport of amino acids in whole blood and plasma of sheep. Am. J. Physiol., 239, E242-E247.

Katz, M.L. and Bergman, E.N., 1969. Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. Am. J. Physiol., 216, 946-952.

Koeln, L.L., Schlagheck, T.G. and Webb, Jr., K.E., 1993. Amino acid flux across the gastrointestinal tract and liver of calves. J. Dairy Sci., 76, 2275-2285.

Lien, K.A., Sauer, W.C. and Fenton, M., 1997. Recovery and composition of crude mucus in ileal digesta of pigs fed a protein-free diet. Z. Ernahrungswiss 36, 182-190.

Low, A.G., 1980. Nutrient absorption in pigs. J. Sci. Food Agric., 31, 1087-1130.

Rerat, A., Simoes Nunes, C., Mendy, F. and Roger, L., 1988. Amino acid absorption and production of pancreatic hormones in non-anaesthetized pigs after duodenal infusions of a milk enzymic hydrolysate or free amino acids. Br. J. Nutr., 60, 121-136.

Rerat, A., 1990. Absorption of nitrogen and amino acids from exogenous (fish meal proteins) or endogenous sources in the pig. Pig News Info., 11, 173-180.

Sarwar, G., Peace, R.W., Botting, H.G. and Brule, D., 1989. Digestibility of protein and amino acids in selected foods as determined by a rat balance method. Plants Foods Human Nutr., 39, 23-32.

Sauer, W.C. and Ozimek, L., 1986. Digestibility of amino acids in swine: results and their practical applications. A review. Livest. Prod. Sci. 15, 367-388.

Sedgwick, G.W., Fenton, T.W. and Thompson, J.R., 1991. Effect of protein precipitating agents on the recovery of plasma free amino acids. Can. J. Anim. Sci., 71, 953-957.

Statistical Analysis System. 1990. SAS/STAT® User's Guide (Release 6.08). SAS Inst. Inc., Cary, NC.

Souffrant, W.B., 1991. Endogenous nitrogen losses during digestion in pigs. In: M.W.A. Verstegen, J. Huisman and L.A. den Hartog (Eds.), Digestive Physiology In Pigs: Endogenous Losses During Digestion In Pigs. Proc. 5th Int. Symp. On Digestive Physiology In Pigs. EAAP Publication no. 54, Pudoc, Wageningen. pp. 147-166.

Steel, R.G.D. and Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Publishing Co., New York.

van Kleef, D.J., 1993. A new system for continuous intravenous infusion of pigs. Lab. Anim. 30, 75-78.

van Leeuwen, P., Leuvenink, H.G.D., Haasbroek, W.M., Priem, G., Bosch, M. and van Kleef, D.J., 1995. A portal-vein-catheterization technique in pigs and sheep, and postprandial changes of pO_2, pCO_2, pH, urea, ammonia, and creatinine and proteins in portal and arterial blood measured in pigs. J. Anim. Physiol. a. Anim. Nutr., 73, 38-46.

van Leeuwen, P., 1997. Personal communication.TNO-Nutrition and Food Research Institute, Department of Animal Nutrition and Meat Technology (ILOB), Wageningen, The Netherlands. .

Webb, Jr., K.E., 1990. Intestinal absorption of protein hydrolysis products: A review. J. Anim. Sci., 68, 3011-3022.

Webb, Jr., K.E. and Bergman, E.N., 1991. Amino acid and peptide transport across the intestine. In: T. Tasada, Y. Sasaki and R. Kawashima (Eds.), Physiological Aspects of Digestion and Metabolism In Ruminants. Proceedings of the 7th International Symposium on Ruminant Physiology. Academic Press, San Diego, CA. pp. 111-128.

Wu, G., Borbolla, A.G. and Knabe, D.A., 1994. The uptake of glutamine and release of arginine, citrulline and proline by the small intestine of developing pigs. J. Nutr., 124, 2437-2444.

Yen, J.T. and Killefer, J., 1987. A method for chronically quantifying net absorption of nutrients and gut metabolites into hepatic portal vein in conscious swine. J. Anim. Sci., 64, 923-934.

Young, J.D., and Ellory, J.C., 1977. Substrate specificity of amino acid transport in sheep erythrocytes. Biochem. J., 162, 33-38.

General Discussion

Introduction

The focus of this thesis is to further develop the relationship between the amino acid composition of endogenous secretions to that of endogenous protein recovered from the ileum of pigs. A better understanding of the factors that affect losses of amino acids from the small intestine is important in terms of maintaining the homeostatic balance of protein metabolism in pigs. Endogenous protein secretions which are not subsequently digested and reabsorbed in the small intestine of the pig constitute a substantial proportion of nitrogen excreted in faecal and urinary waste (Huisman et al. 1993). The burden of nitrogen from animal waste placed on agricultural land is becoming an environmental concern in many areas of the world (Tamminga et al. 1995). Dietary formulations for pigs should provide adequate, but not excessive, levels of amino acids for optimum performance. Better dietary formulations would also lower feed costs and reduce the environmental impact of high levels of nitrogen in feces and urine. In this context, diets for pigs should be formulated on the basis of ileal rather than fecal digestibilities of protein and amino acids because of the modifying effect of microflora in the hind gut (Sauer and Ozimek, 1986). Furthermore, accurate estimates of true ileal amino acid digestibilities (apparent values corrected for recovery of endogenous amino acids) should be used for formulation of diets for pigs in order to more accurately determine the supply of dietary protein. Reliable estimates of ileal recoveries of endogenous amino acids are necessary if the growth performance of pigs is to be maintained while concomitant formulations reduce the level of nitrogen in diets. To address this problem the homoarginine ratio method was evaluated as a new approach to determine endogenous amino acid recoveries in pigs. The effect of soybean trypsin inhibitors and the physiological development of the gastrointestinal tract of newly weaned piglets supplemented with a commercial protease were the factors studied using the aforementioned method. As an alternative to determining the ileal recovery of protein, exchange of amino acids into and out of the portal vein blood of fed versus unfed pigs was measured. This was done to obtain further insight into the absorption of dietary and endogenous protein from the gastrointestinal tract of pigs.

Evaluation and use of the homoarginine ratio method

There have been a number of different methods used to determine the recovery of endogenous nitrogen and amino acids in the ileal digesta of pigs (Nyachoti et al., 1997). These include feeding nitrogen-free diets (e.g. de Lange et al., 1989), peptide alimentation and synthetic amino acid diets (Butts et al., 1993), regression analyses

(Furuya and Kaji, 1986), ^{15}N-leucine, ^{15}N-isoleucine and ^{15}N-isotope dilution techniques (de Lange et al., 1992), ^{13}C-label (Arentson and Zimmerman, 1995) and the homoarginine technique (Barth et al., 1993; Roos et al., 1994). Each of these methods has limitations in terms of their application for determining quantitative estimates of endogenous nitrogen and amino acid recoveries in pigs, especially in studies investigating the effects of antinutritional factors. Antinutritional factors such as Kunitz trypsin inhibitors (Barth et al., 1993) and lectins (Schulze et al., 1995) can be used to increase the amount of endogenous nitrogen leaving the small intestine of pigs. Nevertheless, it is noteworthy that the qualitative estimates of endogenous recoveries of amino acids which have been reported for similar feedstuffs using the different techniques have, more or less, given similar results. For instance, Roos et al. (1994) reported nitrogen digestibilities of 93.5 and 97.6% in the distal region of the small intestine of pigs fed casein labelled with ^{15}N and homoarginine, respectively. They further suggested that the estimate for ^{15}N was underestimated because of recycling of the label in endogenous protein. Chung and Baker, (1992) reported essentially 100% digestibility of amino acids in pigs fed a cornstarch-based diet with casein or an equivalent mixture of crystalline amino acids. Considering the limitations of these techniques, a relationship clearly exists between the recovery of endogenous amino acids and the digestive dynamics of the pig and this may also apply to other animals. It is now well recognized that endogenous recoveries of amino acids can be ascribed to consist of a basal or intrinsic level that is independent of diet (Nyachoti et al., 1997). These basal recoveries are similar to estimates obtained in pigs fed protein-free diets or those determined using the regression analysis method. Ileal recoveries of endogenous amino acid above the basal levels are dependent on extrinsic factors in the diet and physiological state of the pig. With respect to the endogenous recoveries reported in Chapters 1 and 4 there are no directly comparable estimates in the literature that can be used to evaluate the homoarginine ratio method. The "direct" estimates of the homoarginine ratio method were calculated from the flow of amino acids at the distal ileum and the ratio of homoarginine to amino acid concentrations in guanidinated protein test meals and ileal digesta of the pigs as suggested by Siriwan et al. (1994). However, these direct estimates can be compared to "indirect" estimates of endogenous amino acid recoveries as suggested by Marty et al. (1994). They determined true ileal amino acid digestibilities in pigs fed soybean products based on the composition of endogenous protein measured in pigs fed a protein-free diet and parenterally infused with a balanced amino acid solution (de Lange et al., 1989). Therefore, the data of Chapters 1 and 4 were recalculated using the indirect approach as described by Marty

et al. (1994) and compared to the values previously determined by the direct (homoarginine ratio method) approach.

For the indirect approach, total flow (g/kg dry matter intake) of lysine $(Lys)_{flow}$ appearing at the distal ileum of the pigs was calculated using the concentration of lysine (Lys) in digesta and chromic oxide (Cr) as an indigestible marker, as follows:

$$(Lys)_{flow} = (Lys)_{digesta} \times Cr_{diet} / Cr_{digesta}.$$

Table 1.

Endogenous amino acids, determined by the Indirect[a] approach, recovered (g/kg dry matter intake) from the distal ileum of growing pigs fed unprocessed (UGM) and autoclaved (AGM) Nutrisoy guanidinated protein test meals.

	Indirect[b]		
	UGM	AGM	SE[c]
Total of the amino acids[d,e]	37.67	24.22	3.65
Indispensable			
Arginine[d,e]	1.62	1.05	0.15
Histidine[e]	0.82	0.53	0.08
Isoleucine[e]	1.62	1.05	0.15
Leucine[d,e]	2.69	1.75	0.25
Lysine[d,e]	2.17	1.41	0.20
Methionine[d]	0.80	0.52	0.07
Phenylalanine[d,e]	2.87	1.87	0.27
Threonine[d,e]	3.18	2.07	0.30
Valine[d,e]	2.45	1.59	0.23
Dispensable			
Alanine[d,e]	2.27	1.48	0.21
Aspartate + Asparagine[d,e]	3.97	2.58	0.38
Cysteine	1.19	0.51	0.12
Glutamate + Glutamine[d,e]	4.62	3.01	0.44
Glycine[d,e]	3.27	2.12	0.31
Serine[d,e]	2.54	1.65	0.24
Tyrosine[d,e]	1.59	1.03	0.15

[a] Indirect estimates were calculated by reference to flow of amino acids relative to lysine according to Marty et al. (1994) based on the composition of endogenous protein (de Lange et al., 1989).

[b] Means in the same row showed a trend to be different ($P < 0.06$).

[c] Standard error of the mean (n = 6).

[d] Means for endogenous recoveries of amino acids, determined by the Indirect approach, for the unprocessed Nutrisoy guanidinated protein test meals differed ($P < 0.05$) from estimates determined by the direct approach (homoarginine ratio method).

[e] Means for endogenous recoveries of amino acids, determined by the Indirect approach, for the autoclaved Nutrisoy guanidinated protein test meals differed ($P < 0.05$) from estimates determined by the direct approach (homoarginine ratio method).

The apparent digestibility of homoarginine $(AD)_{homo}$ was assumed to be the true ileal digestibility of lysine, as proposed by Hagemeister and Erbersdobler (1985), as follows:

$$AD_{homo} = [\ (homo)_{diet} - (Homo)_{flow} / (homo)_{diet}\ ] \times 100.$$

Endogenous flow of lysine $(EndLys)_{flow}$ was calculated as described by Marty et al. (1994), as follows:

$$(EndLys)_{flow} = (Lys)_{flow} - [\ (Lys)_{diet} - (AD)_{homo} \times (Lys)_{diet} / 100\ ].$$

The endogenous flow of the remaining amino acids $(EndAA)_{flow}$ were then calculated relative to $(EndLys)_{flow}$ based on the composition of endogenous protein in ileal digesta collected from pigs fed protein-free diets and administered a balanced intravenous infusion mixture of amino acids (de Lange et al.,1989) as follows:

$$(EndAA)_{flow} = [\ AA_{literature} / Lys_{literature}\ ] \times (EndLys)_{flow}.$$

Endogenous amino acid recoveries determined by the "indirect" approach for growing pigs fed guanidinated Nutrisoy high or low in soybean trypsin inhibitors (Chapter 1) and for piglets fed protease-treated soybean meal (Chapter 4) are presented in **Table 1** and **2**, respectively.

With a few exceptions the estimates of endogenous amino acid recoveries determined by the indirect approach were higher ($P < 0.05$) than those of the direct approach for the pigs fed the guanidinated Nutrisoy protein test meals (Chapter 1). The difference between estimates of the two approachs was particularly high for the autoclaved Nutrisoy. In this respect, the effect of the high versus low concentration of soybean trypsin inhibitors was more apparent when comparing estimates of the direct approach (homoarginine ratio method) reported in Chapter 1. Indeed, estimates determined by the indirect approach only showed a trend ($P < 0.06$) to be different because they were determined on the basis of a calculated flow of endogenous lysine. On the other hand, estimates for the direct and indirect approachs were usually similar ($P > 0.05$) for the piglets (Chapter 4). The indirect approach has a number of limitations. Firstly, endogenous protein recovered from the ileum of pigs fed the protein-containing test meal is assumed to have an amino acid composition similar to that recovered in pigs fed protein-free diets. As previously pointed out, soybean trypsin inhibitors (Chapter 1), physiological development of piglets (Chapter 3 and 4) and bacteria in the small intestine (Chapter 5) have an affect on the amino acid composition of endogenous protein recovered from the ileum of pigs. Therefore, estimates using the indirect approach are based on basal amino acid levels and do not take into account the specific recoveries due to dietary or physiological factors. Secondly, endogenous amino acid recoveries determined by the indirect approach are based only on the flow of

endogenous lysine. In pigs fed guanidinated protein test meals the flow of amino acids are unlikely to be similar to that calculated by reference to lysine flow in pigs fed a protein-free diet. As previously mentioned, dietary and physiological factors will alter the

Table 2.
Recoveries (g/kg dry matter intake) of endogenous amino acids in ileal digesta of newly weaned piglets[a] determined by the Indirect[b] approach.

	Control meals		Protease-treated meals		
Item	SBM	CI-SBM	PS-SBM	PI-SBM	SEM[c]
Total of the amino acids[d]	26.6	31.2	36.4	22.7	3.6
Indispensable					
Arginine[d]	1.2	1.4[e]	1.6[e]	1.0	0.1
Histidine	0.6[e]	0.7[e]	0.8[e]	0.6	0.1
Isoleucine[d]	1.1	1.4	1.6	1.0[e]	0.2
Leucine[d]	2.0[e]	2.3[e]	2.7[e]	1.6	0.2
Lysine[d]	1.5[e]	1.9[e]	2.2[e]	1.3	0.2
Methionine	0.6	0.7	0.7	0.5[e]	0.2
Phenylalanine[d]	2.1[e]	2.4[e]	2.8[e]	1.7	0.2
Threonine[d]	2.2	2.6	3.1	2.0[e]	0.3
Valine[d]	1.7	2.1	2.4	1.5[e]	0.2
Dispensable					
Alanine[d]	1.6	1.9	2.3	1.4[e]	0.2
Aspartate + Asparagine[d]	2.9[e]	3.3[e]	3.9[e]	2.4	0.3
Cysteine	0.4	0.4[e]	0.4[e]	0.5[e]	0.3
Glutamate + Glutamine[d]	3.4[e]	3.8[e]	4.6[e]	2.8[e]	0.4
Glycine[d]	2.4	2.8	3.2	1.9[e]	0.3
Serine[d]	1.8	2.1[e]	2.5[e]	1.6	0.2
Tyrosine[d]	1.1	1.4	1.6	0.9[e]	0.2

[a] For description of the diets fed to the piglets see the Materials and Methods section of Chapter 3.

[b] See footnote a of Table 1.

[c] Standard error of the mean (n = 6).

[d] At least one of the means in the same row determined by the Indirect approach was different ($P < 0.05$).

[e] Mean was different ($P < 0.05$) from corresponding estimate determined by the direct approach (homoarginine ratio method; Chapter 4). Indirect estimates for PI-SBM were compared to recalculated values; Appendix 1, Chapter 5.

relative flow of individual amino acids to the same extent that flow of endogenous nitrogen is affected by antinutritional factors (Nyachoti et al., 1997). Differences in apparent ileal amino acid digestibilities due to differences in diet and dietary factors are well documented (Sauer and Ozimek, 1986; Sauer and de Lange, 1992). In this respect, endogenous amino acid recoveries in the growing pigs fed a diet differing in concentration of soybean trypsin inhibitors (Chapter 1) were usually overestimated by the indirect approach and underestimated by the direct approach. However, as a qualitative method the difference of endogenous amino acid recoveries due to the content of soybean trypsin inhibitors was more apparent for the direct (homoarginine ratio method) than for the indirect approach.

The homoarginine ratio method may be the best approach currently available to determine qualitative estimates of ileal recovery of endogenous amino acids in pigs fed different dietary protein sources. Further research is needed to determine if the homoarginine ratio method can be used for quantitative estimates of endogenous amino acid recoveries in pigs. In particular, a guanidination procedure for processing large batches of dietary protein needs to be developed. This also applies to a method for removing methylisourea after guanidination that ensures the subsequent total recovery of the soluble portion, especially amino acids of the test protein. The proportion of lysine residues converted to homoarginine in the different protein fractions of plant-based protein sources should be assessed to test whether the guanidination process is random or selective. On this basis, optimum conditions of guanidination can be determined for the various protein sources commonly used in diets for pigs. Finally, further studies are needed on the effect of homoarginine instead of lysine residues in guanidinated protein on the digestion and absorption of dietary and endogenous protein. This information will facilitate the application of the homoarginine ratio method as a quantitative approach in studies with pigs as well as other animals.

Bacteria in the small intestine of pigs

The bacterial contribution of amino acids presented in Chapter 5 suggest that the enterobacteriaceae change both the proportion and composition of amino acids recovered in ileal digesta of pigs. The enterobacteriaceae act as a nitrogen-sink by utilizing available protein of dietary and endogenous origin for growth. This prevents the digestion and absorption of a substantial proportion of available protein and is, therefore, important in terms of the metabolic homeostasis of the pig. The proportion of endogenous amino acids and nitrogen recovered from the ileum of piglets after weaning (Chapter 5), contributed by the endogenous secretions and bacteria are presented in

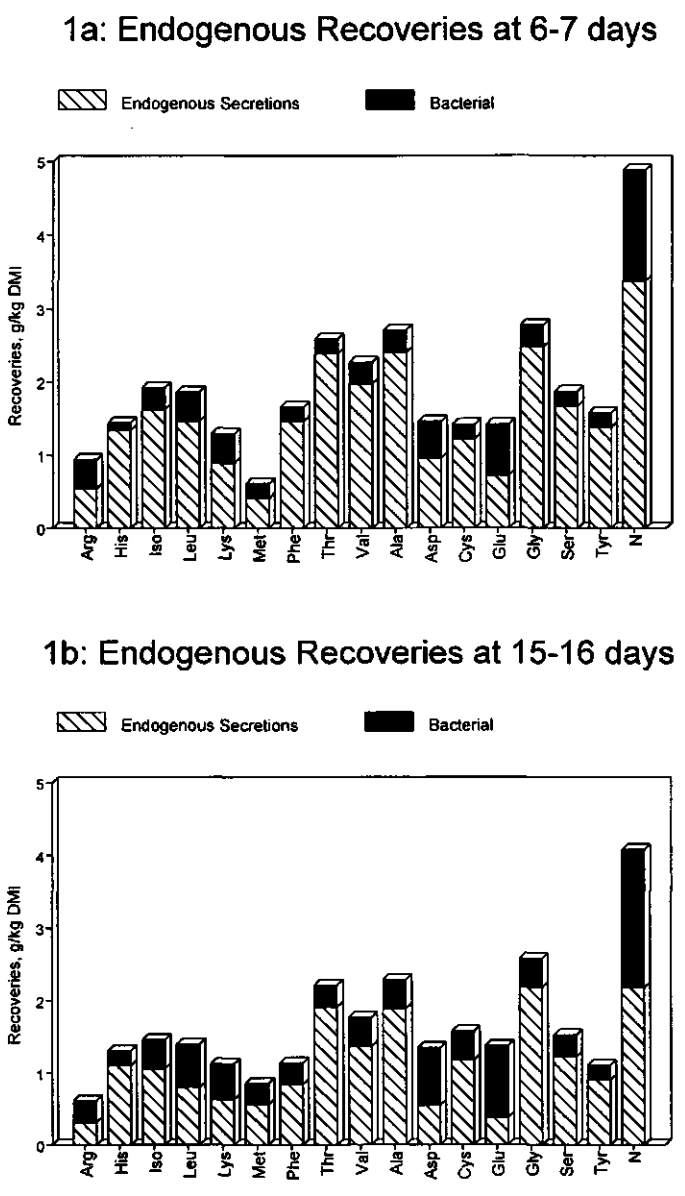

Figure 1.
Contribution of endogenous secretions and bacteria to the ileal recoveries of endogenous amino acids and nitrogen of piglets 6 to 7 (a) and 15 to 16 (b) days after weaning.

Figure 1a and **b**. The bacterial contribution of all amino acids and nitrogen increased from 6 to 7 days after weaning compared to 15 to 16 days after weaning. The metabolic cost, in terms of dietary energy and protein lost to the growing bacterial-flora in the small intestine of piglets after weaning, can be estimated (Chapter 5). Based on the proportion of bacterial to total nitrogen in ileal digesta (Chapter 5) and nitrogen apparent digestibilities (Chapter 3) then 5.3 and 4.9% of the dietary protein intake of the piglets was lost to the bacterial nitrogen-sink at 6 to 7 and 15 to 16 days after weaning, respectively. It is interesting to note that these estimates are similar, especially when the improvement in apparent ileal amino acid digestibilities between the two time periods is considered. The growth of the bacterial-flora in the small intestine coincided with considerable increase in the level of dietary protein intake and an improvement in the ability of the piglets to digest and absorb that protein. Thus, the enterobacteriaceae in an opportunistic manner may be partly compromising the digestive capacity of the piglets. On the other hand, bacterial protein also represents an energy cost to the pig. This can be estimated by assuming that bacterial protein comes entirely from endogenous secretions which have to be subsequently replaced. It was also assumed that 25% of the daily metabolizable energy maintenance requirement (460 KJ/kg$^{0.75}$ body weight; Agricultural Research Council, 1981) of the piglets was associated with the gastrointestinal tract. Furthermore, 4.5 KJ of metabolizable energy are needed to synthesize a gram of protein (Webster, 1981). The estimated energy cost would be 2.4 and 4.1% of the energy expenditure of the gastrointestinal tract of the piglets at 6 to 7 and 15 to 16 days after weaning, respectively. These would be minimum estimates as they do not account for the energy costs of protein turnover or associated metabolic activities such as substrate cycling and ion transport. In this context, the energy expenditure associated with the growth of the enterobacteriaceae increased almost twofold in the short time between the two measurement periods. Of course, these are only approximations but they do indicate a metabolic cost to the pig, that apparently increases in conjunction with growth of the enterobacteriaceae.

Interestingly, the results of Chapter 5 might explain the mode of action for improving feed efficiency and growth in pigs supplemented with antibiotics such as Carbadox (Yen and Nienaber, 1992) and Virginiamycin and Spiramycin (Dierick et al., 1986) or growth-promoting agents such as organic acids (Gabert and Sauer, 1994). The mode of action of these additives has never been fully elucidated, although, a relationship between the alteration of intestinal bacteria and improved nutrient retentions has been postulated. Results of Chapter 5 indicate that a minor decrease or shift in the bacterial flora within the small intestine of pigs, because of supplementation of these

additives, would result in noticeable differences in digestibilities and growth performance. The greatest benefit of these additives was seen in piglets soon after weaning (Gabert and Sauer, 1994) or associated with a reduction in the energy expenditure of the gastrointestinal tract (Yen and Nienaber, 1992) and increased protein digestion (Dierick et al., 1986). Based on the previously discussion, the benefit of these additives are to reduce protein losses by limiting the fixation of protein in the growing bacterial-flora. The associated sparing effect of a reduction in the loss and subsequent resynthesis of endogenous protein increases the available energy for productive metabolic work.

For the most part, the discussion regarding bacteria in the small intestine has been of a negative nature. A synergism exists between enterobacteriaceae and the pig that could be developed for economic and environmental benefits. Bacteria provided with a fermentable carbohydrate source and urea synthesize protein (Mosenthin et al., 1992) that could be potentially available to the pig. Development of nutritional (i.e., inoculation of genetically engineered bacterial species) or pharmacological (i.e., antibiotics against specific bacterial species) strategies that increase this availability of bacterial protein for digestion and absorption could reduce the current dietary protein requirements of pigs. Manipulating the bacterial-flora of the small intestine also has the potential to balance diets for limiting amino acids. The presence of nitrogen-fixing bacteria in the small intestine of pigs is a potential non-dietary source of metabolic nitrogen (March, 1979). The interrelationship between enterobacteriaceae and the pig warrants further study.

Contribution of amino acids from endogenous secretions

With the exception of the present study (Chapter 5) there are no reports which give comparison of the amino acid composition of ileal endogenous amino acids (corrected for bacterial contributions) with those of endogenous secretions. Pancreatic and small intestinal secretions make the largest non-bacterial contribution to total endogenous nitrogen secretion at 17 and 57%, respectively (Souffrant, 1991). In this context, the composition of protein-bound amino acids in pancreatic (Gabert et al., 1996) and mucus (Lien et al., 1997) secretions were compared to those of endogenous secretions recovered in ileal digesta of piglets after weaning (Chapter 5). Differences in the composition are presented in **Figure 2a** and **b**. The percentage composition of most of the amino acids in pancreatic and mucus protein was lower than those recovered as endogenous protein in ileal digesta of the piglets. These differences in composition would be from other endogenous sources such as sloughed epithelial cells

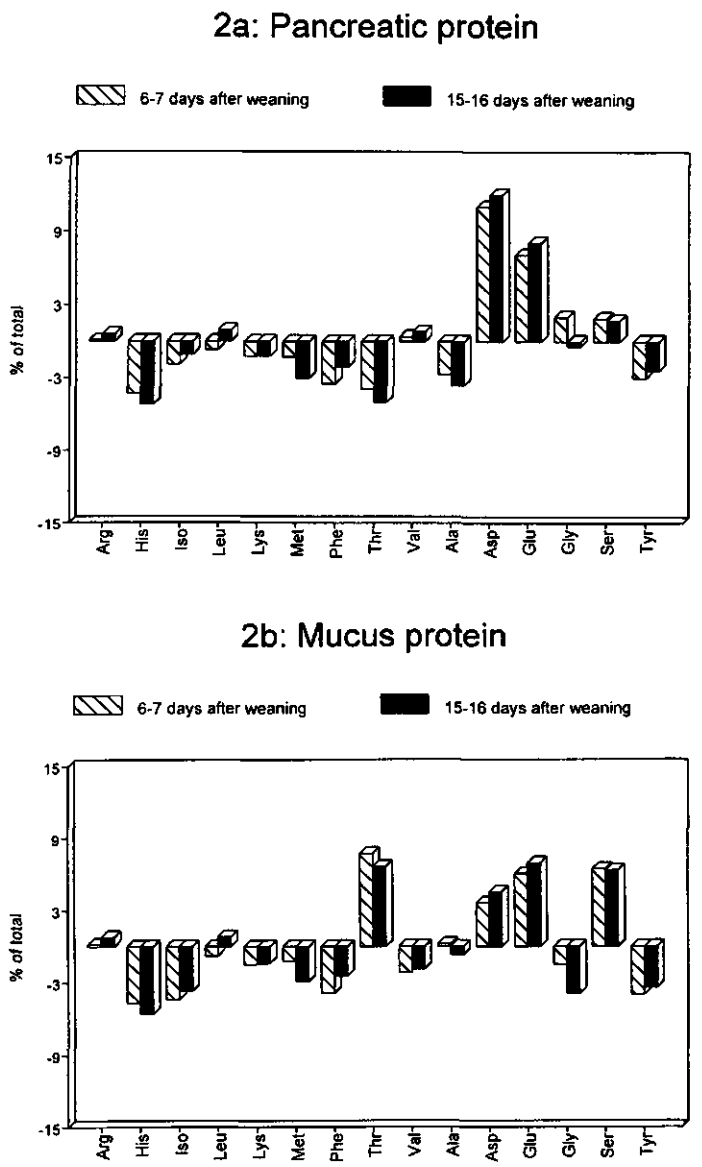

Figure 2.
Difference in the composition (expressed as a percentage of total amino acids) of protein-bound amino acids of pancreatic (a) and mucus (b) secretions from that of ileal recoveries of endogenous amino acids (corrected for bacterial contributions) in piglets 6 to 7 and 15 to 16 days after weaning.

and gastric, bile and intestinal secretions. The notable exceptions were the high concentrations of aspartate and glutamate in pancreatic protein. They have the highest concentrations of all amino acids in amylase and make a large contribution to the composition of trypsin (Gabert et al., 1996). Moreover, a high level of secretion of amylase and trypsin would be expected in piglets which are fed cornstarch-based soybean meal diets (Chapter 3). Presumably, these enzymes must be resistant to proteolytic degradation to remain active in the lumen of the small intestine and, therefore, can constitute a large proportion of ileal recovery of endogenous protein. On the other hand, the concentration of threonine and serine as well as aspartate and glutamate were higher in mucus protein than those of endogenous protein recovered from the ileal digesta of the piglets. Threonine and serine are attachment sites for the oligosaccharide chains in the "native" protein region of the glucoproteins of mucus (Neutra and Forstner, 1987; Dekker, 1990). In this respect, they are resistant to degradation. It is interesting that the difference in the composition of both pancreatic and mucus protein from that of endogenous protein (corrected for bacterial contribution) recovered from the piglets did not change much between 6 to 7 and 15 to 16 days after weaning. This suggests that recoveries of amino acid from pancreatic and mucus protein would, more or less, be similar if expressed on the basis of dietary intake. Whereas, the bacterial contribution of amino acids to endogenous protein recovered from the ileum of the piglets increased with time after weaning. Apparently, the modifying effect of bacteria in the small intestine on the composition and quantity of endogenous protein recovered from the ileum of pigs is substantial and should be investigated further.

General conclusions

It can be concluded from the previous chapters that:

- the homoarginine ratio method is an effective approach to determine ileal recovery of endogenous amino acids and true digestibilities of protein sources fed to pigs,
- a method of guanidinating dietary protein (converting lysine to the synthetic derivative homoarginine) that avoids the losses of soluble carbohydrate and amino acids needs to be developed,
- the *in vitro* pretreatment of soybean meal with protease increases protein solubility and reduces the concentration of soybean trypsin inhibitors,

- treatment of soybean meal with protease, either as a topical spray or by pre-feeding incubation, had no effect on apparent or true ileal digestibilities of amino acids in newly weaned piglets,
- amino acid digestibilities of soybean meal fed to piglets were considerably lower 6 to 7 than 15 to 16 days after weaning. This suggests a rapid adaptive improvement in the digestive and absorptive capacity of the piglets after weaning,
- lower ileal recoveries of endogenous branched-chain and aromatic amino acids from the piglets, 18 compared to 9 days after weaning, suggests dietary change and/or age-dependent adaptive increase in secretion of pepsin and pancreatic proteases,
- pre-feeding incubation of soybean meal with protease increased the bacterial contribution of arginine, phenylalanine, serine and the branched-chain amino acids to total recoveries in ileal digesta of newly weaned piglets,
- the bacterial contributions to total and endogenous recoveries of nitrogen and amino acids in ileal digesta increases with time after weaning,
- the enterobacteriaceae act as a nitrogen-sink by assimilating available protein, thereby, making it unavailable for digestion and absorption by the pig,
- exchange of amino acids into and out of the portal vein blood of growing pigs, 4 hours after receiving a meal, was quantitatively higher in the form of plasma peptides than plasma free amino acids. Red blood cells also probably have a role in the exchange of amino acids,
- a large proportion of dietary amino acids were exchanged into the portal vein blood of the fed pigs in the form of plasma peptides. In this respect, difference in the digestibility of dietary protein may, in part, be dependent on the proportion of amino acids absorbed as peptides.

Literature Cited

Agricultural Research Council. 1981. The nutrient requirements of pigs. Agricultural Research Council, Commonwealth Agricultural Bureau, Slough, UK.

Arentson, R.A., and D.R. Zimmerman. 1995. True digestibility of amino acids and protein in pigs with ^{13}C as a label to determine endogenous amino acid excretion. J. Anim. Sci. 73, 1077-1085.

Barth, C. A., B. Lunding, M. Schmitz and H. Hagemeister. 1993. Soybean trypsin inhibitor(s) reduce absorption of exogenous and increase loss of endogenous protein in miniature pigs. J. Nutr. 123, 2195-2200.

Butts, C. A., P.J. Moughan, W.C. Smith and D.H. Carr. 1993. Endogenous lysine and other amino acid flows at the terminal ileum of the growing pig (20 kg bodyweight): the effect of protein-free, synthetic amino acid, peptide and protein alimentation. J. Sci. Food Agric. 61, 31-40.

Chung, T.K., and D.H. Baker. 1992. Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. J. Anim. Sci. 70, 3781-3790.

Dekker, J. 1990. Gastric mucin, structure and biosynthesis. PhD. Thesis. Rijksuniversiteit Utrecht, Utrecht, The Netherlands.

Dierick, 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*. Livest. Prod. Sci. 14, 177-193.

Furuya, S., and Y. Kaji. 1986. True ileal digestibility of crude protein and amino acids in protein sources as determined by a regression method for growing pigs. Jpn. J. Zootech. Sci. 57, 859-870.

Gabert, V.M., and W.C. Sauer. 1994. The effects of supplementing diets for weanling pigs with organic acids. A review. J. Anim. Feed Sci. 3, 73-87.

Gabert, V.M., W.C. Sauer, S. Li and M.Z. Fan. 1996. Exocrine pancreatic secretions in young pigs fed diets containing fababeans (*Vicia faba*) and peas (*Pisum sativum*): concentrations and flows of total, protein-bound and free amino acids. J. Sci. Food Agric. 70, 256-262.

Hagemeister, H., and H. Erbersdobler. 1985. Chemical labelling of dietary protein by transformation of lysine to homoarginine: a new technique to follow intestinal digestion and absorption. Proc. Nutr. Soc. 44, 133A.

Huisman, J., M.W.A. Verstegen, P. van Leeuwen and S. Tamminga. 1993. Reduction of N pollution by decrease of the excretion of endogenous N in pigs. In: Nitrogen Flow In Pig Production and Environmental Consequences (Verstegen, M.W.A., L.A. den Hartog, G.J.M. van Kempen and J.H.M. Metz, Eds.), pp. 55-61. Pudoc Scientific Publishers, Wageningen, The Netherlands.

de Lange, C.F.M., W.C. Sauer and W.B. Souffrant. 1989. The effect of 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. 67, 755-762.

de Lange, C.F.M., W.C. Sauer, W.B. Souffrant and K.A. Lien. 1992. ^{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. J. Anim. Sci. 70, 1848-1856.

Lien, K.A., W.C. Sauer and M. Fenton. 1997. Recovery and composition of crude mucus in ileal digesta of pigs fed a protein-free diet. Z. Ernahrungswiss 36, 182-190.

Neutra, M.R., and J.F. Forstner. 1987. In: Physiology of the Gastrointestinal Tract (2nd Ed.). pp 975-1009. Raven Press, New York.

Nyachoti, C.M., C.F.M. de Lange, B.W. McBride and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. Can. J. Anim. Sci. 77, 149-163.

March, B.E. 1979. The host and its microflora: an ecological unit. J. Anim. Sci. 49, 857-867.

Marty, B.J., E.R. Chavez and C.F.M. de Lange. 1994. Recovery of amino acids at the distal ileum for determining apparent and true ileal amino acid digestibilities in growing pigs fed various heat-processed full-fat soybean products. J. Anim. Sci. 72, 2029-2037.

Mosenthin, R., W.C. Sauer and C.F.M. de Lange. 1992. Tracer studies of urea kinetics in growing pigs: I. The effect of intravenous infusion of urea on urea recycling and the site of urea secretion into the gastrointestinal tract. J. Anim. Sci. 70, 3458-3466.

Roos, N., M. Pfeuffer and H. Hagemeister. 1994. Labelling with ^{15}N as compared with homoarginine suggests a lower prececal digestibility of casein in pigs. J. Nutr. 124, 2404-2409.

Sauer, W.C., and L. Ozimek. 1986. Digestibility of amino acids in swine: results and their practical application. A review. Livest. Prod. Sci. 15: 367-388.

Sauer, W.C., and C.F.M. de Lange. 1992. Novel methods for determining protein and amino acid digestibilities in feedstuffs. In: Modern Methods in Protein Nutrition and Metabolism. (S. Nissen, Ed.), pp 87-120., Academic Press, San Diego, CA.

Siriwan, P., W.L. Bryden and E.F. Annison. 1994. Use of guanidinated dietary protein to measure losses of endogenous amino acids in poultry. Br. J. Nutr. 71, 515-529.

Schulze, H., H.P. Saini, J. Huisman, M. Hessing, W. van den Berg and M.W.A. Verstegen. 1995. Increased nitrogen secretion by inclusion of soya lectin in the diets of pigs. J. Sci. Food. Agric. 69, 501-510.

Souffrant, W.C. 1991. Endogenous nitrogen losses during digestion in pigs. In: Proceedings of the Vth International Symposium on Digestive Physiology In Pigs. EAAP Publication No. 54. (Verstegen, M.W.A., J. Huisman and L.A. den Hartog Eds.) pp 147-166. PUDOC, Wageningen (Doorwerth), The Netherlands.

Tamminga, S., H. Schulze, J. van Bruchem and J. Huisman. 1995. The nutritional significance of endogenous N-losses along the gastro-intestinal tract of farm animals. Arch. Anim. Nutr. 48, 9-22.

Webster, A.J.F. 1981. The energetic efficiency of metabolism. Proc. Nutr. Soc. 40, 121-128.

Yen, J.T., and J.A. Nienaber. 1992. Influence of Carbadox on fasting oxygen consumption by portal vein-drained organs and by the whole animal in growing pigs. J. Anim. Sci. 70, 478-483.

Summary

Amino acids from endogenous secretions that are not digested and reabsorbed in the small intestine of the pig constitute a substantial proportion of nitrogen excreted in feces and urine. The burden of nitrogen from animal waste placed on agricultural land is becoming an environmental concern in many areas of the world. Dietary formulations for pigs should provide adequate, but not excessive, levels of amino acids for optimum performance. In this context, diets for pigs should be formulated on the basis of true ileal amino acid digestibilities (apparent values corrected for recovery of endogenous amino acids) in order to more accurately determine the supply of dietary protein. Understanding how dietary and physiological factors affect the recovery of amino acids from the ileum is important in terms of maintaining protein homeostasis in pigs. Moreover, reliable estimates of ileal recoveries of endogenous amino acids are necessary if the growth performance of pigs is to be maintained while concomitant formulations reduce the level of nitrogen in diets.

The objectives of this thesis were as follows: (i) to evaluate the homoarginine ratio method as a new approach to determine the ileal recovery of endogenous amino acids in pigs, and (ii) to determine the effect of different dietary concentrations of soybean trypsin inhibitors and (iii) the physiological development of the gastrointestinal tract of newly weaned piglets supplemented with a protease on ileal recovery of endogenous amino acids in pigs. As an alternative to determining the ileal recovery of protein, exchange of amino acids into and out of the portal vein blood of fed versus unfed pigs was measured. This was carried-out (iv) to obtain further insight into the *absorption of dietary and endogenous protein from the gastrointestinal tract of pigs*.

In Chapter 1, the ileal amino acid concentrations of cornstarch-based guanidinated unprocessed (UGM) and autoclaved (AGM) Nutrisoy (defatted soy flour) protein test meals were compared to the respective unguanidinated Nutrisoy diets. Endogenous ileal recoveries and true digestibilities of amino acids were determined in six growing pigs, fitted with a simple T-cannula at the distal ileum, fed the guanidinated protein test meals. The UGM and AGM contained 13.4 (high) and 3.0 (low) g/kg dry matter of soybean trypsin inhibitors (SBTI), respectively. The experiment was a two period cross-over design with each period lasting 15 d. From d 1 to 13 of each period, the pigs were fed the unguanidinated Nutrisoy diets. On d 14 the pigs were fed the guanidinated protein test meals followed by 24-h continuous collection of digesta. Concentrations of crude protein and most of the amino acids in the test meals were higher than in the respective diets. Apparent ileal amino acid digestibilities of the test meals were similar ($P > 0.05$) to reported values for the respective diets and higher ($P < 0.05$) by 22.7 (cysteine) to 61.3 (tyrosine) percentage units for AGM compared to

UGM. The ileal recoveries of endogenous amino acids in AGM-fed pigs were lower ($P < 0.05$) than UGM-fed pigs. Values ranged from -0.10 (arginine) to 0.64 (aspartate + asparagine) and from 0.84 (histidine) to 2.61 (tyrosine) g/kg dry matter intake for AGM- and UGM-fed pigs, respectively. True ileal amino acid digestibilities for AGM were higher ($P < 0.05$) than UGM with differences ranging from 12.7 (tyrosine) to 38.3 (leucine) percentage units. In conclusion, ileal recoveries of endogenous amino acids were increased in pigs fed guanidinated protein test meals with the higher concentration of SBTI. It was further concluded that the homoarginine ratio method was an effective approach to determine qualitative differences in the ileal recovery of endogenous amino acids.

In Chapter 2, an *in vitro* study was undertaken to determine the effect of treating soybean meal with protease at different temperature and pH conditions on the content of total soluble matter and crude protein (CP) and the level of SBTI. Soybean meal was incubated with *Bacillus subtilis* subtilisin-protease (Finnfeeds International Ltd.) at 0, 0.2 or 1.0 mg/g soybean meal in distilled water, adjusted to pH 3, 4.5 or 6, for 16 h at 40 or 50°C. The content of total soluble matter and CP and the level of SBTI were usually similar ($P > 0.05$) for soybean meal treated with protease at 0 or 0.2 mg/g. Protease pretreatment at 1.0 mg/g soybean meal increased ($P < 0.05$) the content of soluble CP and, with the exception of incubation at 50°C and pH 6, lowered ($P < 0.05$) the level of SBTI compared with soybean meal pretreated with protease at 0 or 0.2 mg/g under all conditions of temperature and pH. Based on these results, protease pretreatment at 1.0 mg/g soybean meal has the potential to improve the availability and digestion of soybean meal protein in diets for pigs.

In Chapter 3, apparent ileal digestibilities of amino acids were determined in piglets fed cornstarch based diets with untreated or protease-treated soybean meal as protein source. Twelve piglets, fitted with a modified post valve T-cecum cannula on d 14, 15 and 16 after birth, were weaned at d 20 and assigned to one of four diets according to a two period balanced change-over design. Diets consisted of soybean meal untreated (SBM), incubated (1:2 wt/vol in distilled water adjusted to pH 4.5, for 16 h at 50°C; CI-SBM), sprayed with protease (1 mL/kg of soybean meal; PS-SBM) and incubated with protease as described previously (1 mL/kg of soybean meal; PI-SBM). Apparent CP digestibilities (%) were similar ($P > 0.05$) at 70.4, 72.4, 65.2 and 70.3 for the SBM, CI-SBM, PS-SBM and PI-SBM diets, respectively. Corresponding amino acid digestibilities were also similar ($P > 0.05$), ranging from 62.5, 67.5, 57.9 and 65.0 for alanine to 83.5, 83.4, 78.7 and 84.7 for arginine. Apparent digestibilities were lower ($P < 0.05$) 6 to 7 d after weaning compared with 15 to 16 d after weaning. It was concluded

that protease treatment of soybean meal had no effect on ileal digestibilities of CP and amino acids in newly weaned piglets.

In Chapter 4, endogenous recoveries and true ileal digestibilities of amino acids were determined in piglets fed cornstarch-based diets with untreated or protease-treated soybean meal as protein source, as previously described in Chapter 3. On d 9 of each experimental period, guanidinated protein test meals were fed followed by a 24-h continuous collection of digesta. Recoveries of chromic oxide and dysprosium from the guanidinated meals were 96.0 ± 0.5 and 94.5 ± 1.1 %, respectively. Endogenous amino acid recoveries were similar ($P > 0.05$) for SBM, CI-SBM and PS-SBM but lower ($P < 0.05$) for PI-SBM. Losses of amino acids, particularly homoarginine, during guanidination explain the lower ($P < 0.05$) endogenous amino acid recoveries from the piglets fed PI-SBM. True digestibilities were also lower ($P < 0.05$) for PI-SBM compared with the other meals. Recoveries of endogenous branched-chain and aromatic amino acids were lower ($P < 0.05$) on d 18 than d 9 after weaning, suggesting dietary change- and/or age-dependent adaptive increases in the secretions of pepsin and pancreatic proteases. In conclusion, protease treatment did not improve the true digestibilities of amino acids of soybean meal fed to newly weaned piglets.

In Chapter 5, the bacterial contributions to total and endogenous recoveries of nitrogen (N) and amino acids in ileal digesta of newly weaned piglets were determined. Twelve piglets were fed cornstarch-based diets with untreated or protease-treated soybean meal as protein source, as previously described in Chapter 3. Bacterial material was isolated from pooled subsamples of freeze-dried digesta from piglets within each dietary treatment. The content of diaminopimelic acid (DAPA) in the bacterial isolates and ileal digesta were used to calculate bacterial contributions. The contents of DAPA, N and amino acids in the bacterial isolates were not different ($P > 0.05$) among the diets. Bacterial N contributions were similar ($P > 0.05$); 17.1, 16.4, 14.6 and 21.0% of total N in digesta of the piglets fed SBM, CI-SBM, PS-SBM and PI-SBM, respectively. Bacterial contributions of arginine, isoleucine, leucine, phenylalanine, valine and serine were higher ($P < 0.05$) in ileal digesta of the piglets fed PI-SBM. Contributions to endogenous recoveries of N and amino acids were similar ($P > 0.05$) among the diets. The bacterial contributions of N and amino acids in ileal digesta were lower ($P < 0.05$) on d 6 to 7 than d 15 to 16 after weaning. With the exception of arginine, lysine, methionine and glycine, bacterial contributions to endogenous recoveries were also lower ($P < 0.05$) on d 6 to 7 than d 15 to 16 after weaning. It was concluded that bacterial contributions of N and amino acids in ileal digesta of newly weaned piglets increases after weaning. In this respect, bacteria act as a nitrogen-sink

by assimilating available dietary and endogenous amino acids, thereby, making them unavailable for absorption by the piglets.

In Chapter 6, an experiment was conducted to determine the exchange of amino acids across the portal vein-drained tissue into and out of the plasma, red blood cell and plasma peptide pools of the portal vein blood of growing pigs. The pigs, previously fitted with catheters in an occluded carotid artery and the portal and mesenteric veins, were fed a cornstarch-based diet with wheat gluten as protein source. At 4 (fed) and 28 (unfed) h after receiving a morning meal, simultaneous 8 mL blood samples were taken from the portal vein and carotid artery of each pig. The flow of whole blood and plasma were estimated by the indicator-dilution method, using the infusion of *p*-aminohippuric acid. A second series of blood samples and flow measurements were collected 5 d later. Amino acid concentrations were determined in deproteinized whole blood and plasma and in acid hydrolysates of the plasma. Total exchange of amino acids was higher ($P = 0.02$) in the plasma (55.8 vs 1.9 mmol/h) and plasma peptide (123.2 vs -1.2 mmol/h) pools of fed compared to unfed pigs, respectively. Corresponding total exchange in the red blood cell pool (77.6 vs 6.0 mmol/h) was not statistically significant ($P = 0.29$) because of a large standard error from accumulated analytical variation. The exchange of most of the indispensable amino acids was higher ($P < 0.05$) in the plasma pool of fed than unfed pigs. Corresponding exchange of alanine, asparagine, serine and tyrosine were also higher ($P < 0.05$) in the fed pigs. The exchange of most of the amino acids in the plasma peptide pool of the portal vein blood of fed pigs was usually higher ($P < 0.05$) than that of unfed pigs. Similar amino acid profiles of wheat gluten and the plasma peptide pool indicates that a substantial proportion of dietary amino acids were exchanged and transported in the portal vein blood in the form of plasma peptides. The high content of serine and threonine (intestinal mucus) and glutamate, aspartate and the branched-chain amino acids (pancreatic secretions) suggest that some plasma peptides were of endogenous origin. The exchange of amino acids across the portal vein-drained tissue of pigs is a dynamic process that involves the plasma free amino acid and plasma peptide pools and probably red blood cells.

In summary, the homoarginine ratio method is an effective direct approach to determine the ileal recovery of endogenous amino acids and true digestibilities of protein sources fed to pigs. Treatment of soybean meal with protease had no effect on apparent or true ileal digestibilities of amino acids in newly weaned piglets. Lower apparent ileal amino acid digestibilities of soybean meal fed to piglets 6 to 7 than 15 to 16 d after weaning suggest a rapid adaptive improvement in the digestive and absorptive capacity of the piglets after weaning. Furthermore, lower recoveries of

endogenous branched-chain and aromatic amino acids on d 18 than on d 9 after weaning, suggest dietary change- and/or age-dependent adaptive increases in the secretions of pepsin and pancreatic proteases. The bacterial contributions to total and endogenous recoveries of nitrogen and amino acids in ileal digesta increase with time after weaning. Bacteria act as a nitrogen-sink by assimilating available protein, thereby, making it unavailable for digestion and absorption by the pig. Difference in the digestibility of dietary protein may, in part, be dependent on the proportion of amino acids absorbed as peptides.

Samenvatting

Aminozuren afkomstig van endogene uitscheidingen welke niet verteerd en gereabsorbeerd worden in de dunne darm van het varken dragen substantieel bij aan de stikstof uitgescheiden in mest en urine. De stikstof uit dierlijke mest aangewend op landbouwgrond zorgt in vele delen van de wereld voor een ongewenst hoge milieubelasting. Het aanbod van aminozuren in voer dient daarom nauwkeurig afgestemd te worden op de behoefte van het varken zodat optimale dierprestaties gehandhaafd kunnen blijven zonder dat het milieu overdadig belast wordt. Om de eiwitvoorziening voor het varken nauwkeuriger af te stemmen op de behoefte dienen rantsoenen daarom samengesteld te worden op basis van de ware ileale aminozuurverteerbaarheid (schijnbare waarden gecorrigeerd voor de uitscheiding aan endogene aminozuren). Inzicht in de invloed van voer- en fysiologische factoren op de ileale aminozuuruitscheiding is belangrijk met het oog op het handhaven van een evenwichtig eiwitmetabolisme in varkens. Betrouwbare schattingen van endogene aminozuren in het ileum zijn ook nodig als men de groei van varkens wil handhaven terwijl toch het aanbod van stikstof met het voer gereduceerd wordt.

De doelstellingen van het hier beschreven onderzoek waren: (i) evaluatie van de homo-arginine methode als nieuwe benadering voor de bepaling van endogene aminozuurverliezen in het ileum van varkens, en (ii) bepalen van het effect van verschillende concentraties aan trypsineremmers in het voer op de ileale uitscheiding van endogene aminozuren en (iii) bepalen van het effect op de endogene aminozuur uitscheiding van de fysiologische ontwikkeling van het maagdarmkanaal van pasgespeende biggen die een protease gesupplementeerd rantsoen verstrekt krijgen in het ileum. Als een alternatief voor de bepaling van ileale eiwitverliezen werd de portale verschijning van aminozuren van gevoerde versus niet gevoerde varkens gemeten. Dit werd gedaan om (iv) meer inzicht te krijgen in de absorptie van rantsoen- en endogeen eiwit vanuit het darmkanaal van varkens.

In Hoofdstuk 1 werden in groeiende varkens de ileale aminozuurconcentraties bestudeerd na het voeren van op maiszetmeel gebaseerde geguanidineerde (een behandeling waarbij het eiwitgebonden lysine na reactie met methyl-iso-ureum homoarginine vormt), onbehandelde (UGM), en geautoclaveerde (AGM) Nutrisoy (ontvette soja bloem) rantsoenen vergeleken met de ongeguanidineerde Nutrisoy rantsoenen. Endogene ileale aminozuurverliezen en ware aminozuurverterings-coëfficiënten werden bepaald in zes groeiende varkens voorzien van een T-canule in het distale ileum, gevoerd met de geguanidineerde eenmalig verstrekte testrantsoenen. Het UGM en het AGM bevatte respectievelijk 13,4 (hoog) en 3,0 (laag) g/kg droge stof soja trypsine remmer (SBTI). De proef bestond uit een cross over schema met twee perioden van elk 15 dagen. Van dag 1 tot 13 van elke periode kregen de dieren de

ongeguanidineerde rantsoenen verstrekt. Op dag 14 werden eenmalig de eiwit geguanidineerde testrantsoenen verstrekt waarna gedurende 24 uur de ileale chymus kwantitatief verzameld werd. De concentraties aan ruw eiwit en het merendeel van de aminozuren in de testrantsoenen waren hoger dan in de respectievelijke controle rantsoenen. Schijnbare ileale aminozuurverteerbaarheden van de testrantsoenen waren vergelijkbaar (P>0.05) met in de literatuur vermelde waarden van de respectievelijke rantsoenen en hoger (P<0.05) voor AGM dan voor UGM, varierend van 22,7 (cysteine) tot 61,3 (tyrosine) eenheden. De ileale verliezen aan endogene aminozuren in de AGM gevoerde varkens waren lager (P<0.05) dan in de UGM gevoerde varkens. Waarden varieerden van -0,10 (arginine) tot 0,64 (aspartaat + asparagine) en van 0,84 (histidine) tot 2,61 (tyrosine) g/kg droge stof opname voor respectievelijk de AGM- en de UGM gevoerde dieren. De ware aminozuur verteringscoëfficiënten waren hoger (P<0.05) voor AGM dan voor UGM, met verschillen variërend van 12,7 (tyrosine) tot 38,3 (leucine) eenheden. Geconcludeerd werd dat de ileale endogene aminozuur verliezen hoger waren voor varkens gevoerd met de geguanidineerde testrantsoenen met de hoogste concentratie aan SBTI. Verder werd geconcludeerd dat met de homoarginine ratio methode kwalitatieve verschillen in ileale endogene aminozuurverliezen bepaald kunnen worden.

In Hoofdstuk 2 wordt een *in vitro* experiment beschreven om het effect te bepalen op de hoeveelheid aan totaal oplosbaar materiaal, ruw eiwit (CP) en het SBTI niveau wanneer sojaschroot wordt behandeld met protease bij verschillende temperaturen en verschillende pH waarden. Het sojaschroot werd in gedestilleerd water gedurende 16 uur geïncubeerd met *Bacillus subtilis* subtilisine-protease (Finnfeeds International Ltd.) (0, 0.2 of 1.0 mg /g sojaschroot). Elke concentratie werd geïncubeerd bij een temperatuur van 40 en 50°C en een pH van 3, 4.5 of 6. Het gehalte aan totaal oplosbaar materiaal en ruw eiwit en het SBTI gehalte waren over het algemeen gelijk (P > 0.05) voor sojaschroot behandeld met protease concentraties van 0 of 0.2 mg/g. Behandeling van sojaschroot met protease (1.0 mg/g sojaschroot) verhoogde (P < 0.05), het gehalte aan ruw eiwit. Met uitzondering van incubatie bij 50°C en pH 6 was het SBTI gehalte lager (P < 0.05) na incubatie met 1.0 mg /g dan na incubatie met 0 of 0.2 mg protease per g sojaschroot. Aan de hand van deze resultaten kan worden geconcludeerd, dat behandeling van sojaschroot met protease op een niveau van 1.0 mg/g de beschikbaarheid en de verteerbaarheid van eiwitten in sojaschroot in rantsoenen voor varkens kan verbeteren.

In Hoofdstuk 3 wordt een onderzoek beschreven waarin de schijnbare ileale verteerbaarheid van aminozuren bepaald werd bij biggen, die gevoerd werden met een op maiszetmeel gebaseerd rantsoen met respectievelijk onbehandeld of met protease

behandeld sojaschroot als eiwitbron. Twaalf biggen werden op dag 14, 15 of 16 na de geboorte voorzien van een gemodificeerde "post valve T-caecum". De dieren werden op een leeftijd van 20 dagen gespeend en toegewezen aan één van de 4 rantsoenen volgens een "two-period balanced change-over design". De rantsoenen bevatten onbehandeld sojaschroot (SBM), SBM geïncubeerd met gedestilleerd water (1:2wt/vol bij een pH van 4.5, gedurende 16 uur bij 50°C; CI-SBM), SBM bespoten met protease (1 mL/kg sojaschroot; PS-SBM) of SBM geïncubeerd met protease zoals eerder beschreven (1 mL/kg sojaschroot; PI-SBM). De schijnbare ruw eiwit verteerbaarheid (%) was vergelijkbaar voor de 4 rantsoenen ($P > 0.05$) en was respectievelijk 70.4, 72.4, 65.2 en 70.3 voor SBM, CI-SBM, PS-SBM en PI-SBM. De verteerbaarheid van overeenkomstige aminozuren was op hetzelfde niveau voor de verschillende behandelingen ($P > 0.05$) en varieerde van 62.5, 67.5, 57.9 en 65.0 voor alanine tot 83.5, 83.4, 78.7 en 84.7 voor arginine. De schijnbare verteerbaarheid 6 tot 7 dagen na het spenen was lager ($P < 0.05$) dan 15 tot 16 dagen na het spenen. Geconcludeerd werd dat behandeling van sojaschroot met protease geen effect had op de verteerbaarheid van ruw eiwit en aminozuren in de dunne darm van pas gespeende biggen.

In het onderzoek beschreven in Hoofdstuk 4 werden zowel de endogene verliezen als de ware verteerbaarheden van aminozuren in de dunne darm bepaald in biggen. De dieren kregen op maiszetmeel gebaseerde rantsoenen met als eiwitbron onbehandelde en met protease behandelde sojaschroot zoals eerder beschreven in hoofdstuk 3. Op dag 9 van iedere experimentele periode werden éénmalig testrantsoenen waarvan het eiwit geguanidineerd was toegediend, gevolgd door een kwantitatieve verzameling van chymus gedurende 24 uur. Recoveries van chroomoxide en dysprosium by de geguanidineerde maaltijden bedroegen respectievelijk 96.0 ± 0.5 en 94.5 ± 1.1 % van de opgenomen hoeveelheid. Endogene aminozuurverliezen waren vergelijkbaar ($P > 0.05$) voor rantsoenen SBM, CI-SBM en PS-SBM, maar lager ($P < 0.05$) voor het PI-SBM rantsoen. Verliezen aan aminozuren, met name aan homoargine, gedurende het guanidineren, verklaart het lagere ($P < 0.05$) endogene verlies aan aminozuren voor met PI-SBM gevoerde biggen. In vergelijking met de andere rantsoenen was ook de ware verteerbaarheid van PI-SBM lager ($P < 0.05$). De verliezen aan endogene vertakte en aromatische aminozuren waren lager ($P < 0.05$) op dag 18 dan op dag 9 na het spenen. Dit suggereert een effect van verandering in rantsoen en/of een leeftijdsafhankelijke toename in de secretie van pepsine en pancreas proteasen. Hieruit mag geconcludeerd worden dat protease behandeling de ware verteerbaarheid van aminozuren uit sojaschroot in pas gespeende biggen niet verbetert.

In Hoofdstuk 5 werd de bacteriële bijdrage aan het totale en endogene verlies van stikstof (N) en aminozuren in ileale chymus van pas gespeende biggen onderzocht. Twaalf biggen werden gevoerd met een op maiszetmeel gebaseerd rantsoen met onbehandelde of met protease behandelde sojaschroot als eiwitbron, zoals eerder beschreven in hoofdstuk 3. Submonsters van gevriesdroogde chymus van biggen met dezelfde behandeling werden samengevoegd en uit dit mengmonster werd bacterieel materiaal geïsoleerd. De concentratie di-amino-pimelinezuur (DAPA) in het bacteriële isolaat en in de ileale chymus werd gebruikt om het bacteriële aandeel in de totale en endogene verliezen te berekenen. De DAPA-, stikstof- en aminozuurgehaltes in het bacteriële isolaat verschilden niet ($P > 0.05$) voor de verschillende rantsoenen. De bacteriële stikstofbijdrage was niet significant verschillend ($P > 0.05$) in chymus voor met SBM, CI-SBM, PS-SBM en PI-SBM gevoerde biggen en bedroeg respectievelijk, 17.1, 16.4, 14.6 en 21.0% van het totale stikstofgehalte. De bacteriële bijdrage aan arginine, isoleucine, leucine, fenylalanine, valine en serine was hoger ($P < 0.05$) in chymus van biggen die met PI-SBM gevoerd werden. De bijdrage aan endogene stikstof- en aminozuurverliezen was gelijk ($P > 0.05$) voor de verschillende rantsoenen. Het bacteriële aandeel van stikstof en alle aminozuren in ileaal chymus was lager ($P < 0.05$) op 6 tot 7 dagen dan op 15 tot 16 dagen na het spenen. Het bacteriële aandeel in het endogene verlies, met uitzondering van arginine, lysine, methionine en glycine, was lager ($P < 0.05$) op 6 tot 7 dagen dan op 15 tot 16 dagen na het spenen. Geconcludeerd werd dat de bacteriële bijdrage aan stikstof en aminozuren in ileale chymus bij pas gespeende biggen toeneemt na spenen. Bacteriën gebruiken zowel endogene als uit de voeding afkomstige aminozuren waardoor deze niet meer beschikbaar zijn voor absorptie door de biggen. Dit geldt althans voor bacteriën, die op het eind van de dunne darm nog aanwezig zijn.

In Hoofdstuk 6, wordt een experiment beschreven waarin de hoeveelheid aminozuren, die vanuit het maagdarmkanaal naar het plasma, de rode bloedcellen en de plasmapeptide voorraad in de poortader stroomt, gemeten wordt. Bij de varkens werden catheters in de halsslagader, de poortader en de vena mesenterica ingebracht. Vervolgens werden de varkens gevoerd met een op maïszetmeel gebaseerd rantsoen met tarwegluten als eiwitbron. Op 4 (gevoerd) en 28 (ongevoerd) uur na de ochtendvoeding werd simultaan 8 ml bloed afgenomen uit de poortader en de halsslagader van elk varken. De flow van bloed en bloedplasma werden geschat aan de hand van de indicator verdunnings methode waarbij para-amino hippuurzuur (PAB) geinfuseerd werd. Vijf dagen later werd een tweede serie flow-metingen gedaan en bloedmonsters verzameld. Aminozuurconcentraties werden bepaald in onteiwit bloed en plasma en ook in het zure hydrolisaat van het plasma. In gevoerde varkens was de

toename van aminozuren hoger ($P = 0.02$) in het plasma (55.8 vs 1.9 mmol/h) evenals in de plasma peptidevoorraad (123.2 vs - 1.2 mmol/h). De totale hoeveelheid in de rode bloedcellen werd berekend uit het verschil tussen de aminozuur-concentratie in het bloed en in het plasma. Door de grote standaardfout konden geen significante verschillen ($P = 0.29$) worden aangetoond. De uitwisseling van de meeste essentiële aminozuren was hoger ($P < 0.05$) in de plasmavoorraad van gevoerde dan van ongevoerde varkens. De bijbehorende uitwisseling van alanine, asparagine, serine en tyrosine was eveneens hoger ($P < 0.05$) in de gevoerde varkens. De toename van de meeste aminozuren in de plasma peptidevoorraad van de poortader van gevoerde varkens was meestal hoger ($P < 0.05$) die van ongevoerde varkens. Overeenkomstige aminozuurprofielen van tarwegluten en de plasma peptiden geeft aan dat een substantieel deel van de aminozuren uit het rantsoen werden opgenomen en getransporteerd in het bloed in de vorm van plasmapeptiden. Het hoge gehalte aan serine en threonine (darm mucosa) en glutamaat, aspartaat en de vertakte aminozuren (pancreas excretie) suggereert, dat sommige plasma peptiden van endogene oorsprong zijn. Geconcludeerd werd dat de opname van aminozuren in het portale bloed van varkens een dynamisch proces is waarbij de vrije aminozuren in het plasma en de plasma peptidevoorraad en waarschijnlijk de rode bloedcellen betrokken zijn.

Samenvattend is de homo-arginine ratio methode een effectieve directe benadering voor de bepaling van endogene aminozuurverliezen in de dunne darm van varkens en voor bepaling van de ware verteerbaarheid van eiwitbronnen. Behandeling van sojaschroot met protease had geen effect op de schijnbare of de ware verteerbaarheid van aminozuren in de dunne darm van pas gespeende biggen. Een lagere schijnbare verteerbaarheid van aminozuren uit sojaschroot gevoerd aan biggen 6-7 dagen na het spenen ten opzichte van biggen van 15-16 dagen na het spenen suggereert een snelle adaptieve verbetering in de verterings- en absorptiecapaciteit van biggen na het spenen. Bovendien suggereert een lager verlies van endogene vertakte en aromatische aminozuren op dag 18 na het spenen t.o.v. dag 9 na het spenen een effect van verandering in rantsoen en/of een leeftijdsafhankelijke toename in de secretie van pepsine en pancreas-proteasen. De bacteriële bijdrage aan het totale en endogene verlies aan stikstof en aminozuren in de ileale chymus neemt na het spenen toe in de tijd. Bacteriën assimileren beschikbaar eiwit waarna dit eiwit niet meer beschikbaar is voor vertering en absorptie door het varken zelf. Het verschil in verteerbaarheid van eiwitten tussen de rantsoenen, zou ten dele ook afhankelijk kunnen zijn van het aandeel aminozuren, dat als peptiden geabsorbeerd wordt.

First author publications:

Caine, W.R., W.C. Sauer, S. Tamminga, M.W.A. Verstegen and H. Schulze, 1997. Apparent ileal digestibilities of amino acids in newly weaned piglets fed diets with protease-treated soybean meal. *J. Anim. Sci.* 75: 2962-2969.

Caine, W.R., S. Tamminga, M.W.A. Verstegen, W.C. Sauer and H. Schulze, 1997. Endogenous recoveries and true ileal digestibilities of amino acids in newly weaned piglets fed diets with protease-treated soybean meal. *J. Anim. Sci.* 75: 2970-2979.

Caine, W.R., M.W.A. Verstegen, W.C. Sauer, S. Tamminga and H. Schulze, 1997. Effect of protease treatment of soybean meal on content of total soluble matter and crude protein and level of soybean trypsin inhibitors. *Anim. Feed Sci. Technol.*, In press.

Caine, W.R., W.C. Sauer, M.W.A. Verstegen, S. Tamminga, S. Li and H. Schulze, 1997. Guanidinated protein test meals with higher concentration of soybean trypsin inhibitors increase ileal recoveries of endogenous amino acids in pigs. *J. Nutr.*, In Press.

Caine, W.R., S. Tamminga, W.C. Sauer, M.W.A. Verstegen and H. Schulze, 1997. Bacterial contributions to total and endogenous recoveries of nitrogen and amino acids in ileal digesta of newly weaned piglets fed protease-treated soybean meal. *Livest. Prod. Sci.*, submitted.

Caine, W.R., M.W.A. Verstegen, W.C. Sauer, S. Tamminga and P. van Leeuwen, 1997. Exchange of amino acids and peptides across the portal vein-drained tissue of pigs. To be submitted.

Caine, W.R. and G.W. Mathison, 1992. Influence of the β-adrenergic agonist cimaterol on energetic efficiency and oxygen consumption in liver tissue of growing lambs. *Can. J. Anim. Sci.*, 72:555-568.

Caine, W.R. and G.W. Mathison, 1992. Influence of the β-adrenergic agonist cimaterol on body composition and whole body synthesis and degradation of protein in growing lambs. *Can. J. Anim. Sci.*, 72:569-587.

Coauthored publications:

Li, S., W.C. Sauer and W.R. Caine, 1997. Response of nutrient digestibilities to feeding diets of low and high levels of soybean trypsin inhibitors in growing pigs. *J. Sci. Food Agric.* In press.

Todd, L.K., W.C. Sauer, R.J. Christopherson, R.J. Coleman and W.R. Caine, 1995. The effect of feeding different forms of alfalfa on nutrient digestibility and voluntary intake in horses. *J. Anim. Physiol. a. Anim. Nutr.*, 73:1-8.

Todd, L.K., W.C. Sauer, R.J. Christopherson, R.J. Coleman and W.R. Caine, 1995. The effect of level of feed intake on nutrient and energy digestibilities and rate of feed passage in horses. *J. Anim. Physiol. a. Anim. Nutr.*, 73:140-148.

Dugan, M.E.R., W.C. Sauer, J.M. Dugan, Jr. and W.R. Caine, 1994. Determination of bacterial amino acid contribution to ileal digesta from pigs using ^{35}S and DAPA marker techniques. *J. Anim. Feed Sci.*, 3:149-159.

Imbeah, M., W.C. Sauer and W.R. Caine, 1994. Comparison of the single dose and withdrawal method for measuring the rate of passage of two digestibility markers in digesta collected from the distal ileum and feces in growing pigs. *Anim. Feed Sci. Technol.*, 52:41-50.

Yaremcio, B.J., G.W. Mathison, D.F. Engstrom, L.A. Roth and W.R. Caine, 1991. Effect of ammoniation on the preservation and feeding value of barley grain for growing-finishing cattle. *Can. J. Anim. Sci.*, 71:439-455.

Other publications:

Caine, W.R., W.C. Sauer, S. Tamminga, M.W.A. Verstegen and H. Schulze, 1997. Apparent and true ileal digestibilities of amino acids in newly-weaned piglets fed diets with protease-treated soybean meal. In: *Proceedings of the 7th Symposium on Digestive Physiology In Pigs.* Laplace, J.P., C. Fevrier, A. Barbeau (Ed.). Palais du Grand Large, Saint Malo, The Netherlands. p.359-362.

Caine, W.R., M.W.A. Verstegen, S. Tamminga, W.C. Sauer and H. Schulze, 1997. Bacterial contributions of N and indispensable amino acids to ileal digesta of newly-weaned piglets fed diets with protease-treated soybean meal. In: *Proceedings of the 7th Symposium on Digestive Physiology In Pigs.* Laplace, J.P., C. Fevrier, A. Barbeau (Ed.). Palais du Grand Large, Saint Malo, The Netherlands. p. 454-457.

Caine, W.R., W.C. Sauer, S. Tamminga, M.W.A. Verstegen and H. Schulze, 1997. Apparent and true ileal digestibilities of amino acids in newly-weaned piglets fed diets with protease-treated soybean meal. *Annual Meeting of American Society of Animal Science*, Tennessee, United States. Abstract.

Caine, W.R. and G.W. Mathison, 1987. Growth and carcass performance in wether lambs fed the repartitioning agent cimaterol. *Sixty-sixth Annual Feeders' Day Report.* Department of Animal Science, The University of Alberta, Edmonton. p. 89-90.

Caine, W.R. and G.W. Mathison, 1988. Effects of the repartitioning agent cimaterol on energetic efficiency. *Sixty-seventh Annual Feeders' Day Report.* Department of Animal Science, The University of Alberta, Edmonton. p. 37-38.

About the author

William Robert Caine was born September 23, 1953 in Grande Prairie, Alberta, Canada. In June of 1975, he received a Bachelor of Science Degree in Biology from the Faculty of Science, at the University of Alberta. After graduation, together with his father (Bob) and brother (Russell), he started Caine Cattle Company, a one thousand hectare ranch raising purebred Polled Hereford and Aberdeen Angus cattle. Annual seed-stock production sales at Caine Cattle Company were attended by people from all over the world. Cattle with the CCCH and CCC tattoos have gone into herds in Denmark, Germany, the British Isles, Japan, China, Australia, New Zealand and the United States. William's scientific curiosity caused him to return to the University of Alberta, Department of Animal Science in 1985 for further studies. In October of 1989 he completed a Master of Science degree in Animal Nutrition. His thesis topic was "Energetic Efficiency of Cimaterol-Treated Lambs". In 1994, William was awarded a six month study Fellowship by the Dutch Ministry of Agriculture, Nature Management and Fisheries. From September of 1994 until March of 1995 he conducted experiments at the TNO-Nutrition and Food Research Institute, Department of Animal Nutrition and Meat Technology (ILOB), in collaboration with the Wageningen Institute of Animal Sciences, Department of Animal Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands. This project developed into a PhD. study to investigate endogenous amino acid recoveries in pigs.

William's keen interest in agriculture has made a lasting impression on his three children. His oldest daughter, Jill Allison, graduated in June of 1997 with a Diploma specializing in Animal Sciences, from the Olds Agricultural College in Alberta. She is planning a career as a livestock buyer and broker. His daughter, Jane Arden, is studying towards a Bachelor of Science degree from the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta. His son, Jonathan Adam, is a creative writer and is planning a career in journalism after he graduates from High School in June of 1998.