Factors affecting the nutrient digestibility in weanling and grower pigs

BY

Vijayasmitha Rayadurg

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Dedicated

to

Dr. Hans H. Stein

“Guru Sakshath Parabrahma, Tasmei Sri Guruve Namaha”

Guru is verily the embodiment of God. My pranams to such Guru Deva.

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department

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Hans H. Stein, Ph. D.
Dissertation Advisor Date

_____

Donald L. Boggs, Ph. D.
Head, Department of
Animal and range Sciences Date

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ABSTRACT

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Experiments were conducted with the objective of evaluating factors that affect the nutrient digestibility in weanling and growing pigs. The first study was conducted to elucidate the effect of the antimicrobial carbadox on the porcine small intestinal structure. Pigs on treatment group 1 received a diet without any in-feed antibiotic (AB-). Pigs on treatment group 2 received the same diet with an antibiotic (carbadox) included at 50 ppm (AB+). Regardless of the site of sampling, there was no effect of addition of carbadox to the diet on villus height in the small intestine. Likewise, there were no differences in crypt depth values between sites in the small intestine and between diets. No effects of time, diet, or intestinal location were found for the mitotic index. Four experiments were conducted to measure ileal digestibility coefficients of amino acids using pigs that were equipped with a T – cannula in the distal ileum. Chromic oxide was the inert marker in the diets for all the experiments. In one experiment, the effect of time after weaning on the apparent ileal digestibility of starch and amino acids were assessed in diets containing three different protein sources. The results of this experiment showed no effect of time after weaning on the digestibility of nutrients. In a further experiment, the effect of feed intake on the apparent and standardized ileal digestibility coefficients was measured in an experiment utilizing six growing barrows. Experimental diets was

fed at a level calculated to equal the maintenance requirement of the pig (M1), at two times the maintenance (M2), and at three times the maintenance requirement (M3). Increasing the feed intake increased the apparent ileal digestibility coefficients, but reduced the endogenous losses of amino acids, and therefore, also reduced the standardized ileal digestibility coefficients. The objective of the next experiment was to evaluate the effect of processing on the digestibility of amino acids in blood cell products by grower pigs. Five barrows (initial BW: 41.4 ± 3.1 kg) were used in this experiment and the apparent and standardized ileal digestibility coefficients were measured in three sources of blood cells and in casein. Results of this experiment showed that there is a significant effect of processing on the digestibility of amino acids in blood cells. These results led to the recommendation that peroxide should not be used to process blood cells. The effect of the in-feed acidifier Aciprol on AA digestibility in grower pigs was assessed in a final experiment. Results from this experiment conducted revealed that the positive effects of acidifiers that have been reported in other experiments are not caused by an increase in the ileal digestibility of dietary amino acids.

Key Words: Acidifier, Amino acids, Antimicrobials, Digestibility, Pigs, Processing.

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CHAPTER 1

Introduction

Swine obtain most of their AA requirements from the proteins in feedstuffs they consume. Proteins are considered to be essential nutrients because when swine are fed diets that are deficient in protein they do not grow or reproduce normally (Baker and Spencer, 1983). Amino acids, the components that make up the protein are the key ingredients for normal growth and productivity of animals.

Amino acids are classified as essential, non-essential and conditionally essential based on their requirement and synthesis by the animal (Lewis, 2001). Proteins differ in their quality based on their AA composition. A protein that has a balance of AA among essential AA and between essential and non-essential AA is called an ideal protein (Baker, 1977).

Harper et al. (1970) stated that the departure from the ideal protein pattern will lead to a reduction in animal performance in terms of efficiency with which the AA are used. In practical diets for swine, the AA disproportion of concern is a deficiency of one or more AA. The AA that is present in the least amount relative to its requirement is said to be the first limiting AA. The extent to which an AA is essential to the animal dictates its performance (Lewis, 2001).

Information of limiting AA in feedstuffs is very important to formulate balanced swine rations. The formulation of diets based on AA is a precise approach. The AA

composition of feedstuffs can be determined by chemical procedures. However, absorption and presentation of AA in a form that can be utilized by the animal tissues is the critical criteria. Several reviews have been published on this topic (Tanksley and Knabe, 1984; Lewis and Bayley, 1995).

Amino acid digestibility is the most important determinant of AA utilization in feedstuffs by pigs (Fan, 1994). Ileal digestibility of AA is important in swine nutrition because of the presence of variation in quality of feed proteins between different feedstuffs and within same feedstuff (Lewis, 2001). Digestibility of AA is also affected by processing, heat treatment, fiber content, quality of the feed protein, and anti nutritional factors in the feed (Jansman et al., 1993).

Post weaning growth check in piglets happens due to the stress of weaning and change in diet is a production penalty in the swine industry. Changes occur in gut structure after weaning leading to a reduction in digestive and absorptive ability of the small intestine (Cera et al., 1988). Feeding sub therapeutic levels of antimicrobials to pigs has been shown to enhance voluntary feed intake and growth promotion (Cromwell, 2001). Possible modes of action of antimicrobials are thought to be of metabolic, nutritional, and disease control effect (Hays, 1978).

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CHAPTER 2

Factors affecting amino acid digestibility by swine: A literature review

1. Introduction

In monogastric animals, digestibility of limiting AA is the key issue in determining the nutritive value of protein (Mosenthin et al., 2000). The difference in apparent ileal digestiblity coefficients (AID) of AA between feedstuffs has been reported to be due to variations in processing conditions, inherent dietary factors, variety of grain, fertilizer application, and environmental conditions (Sauer and Ozimek, 1986; Mosenthin et al., 1997). For similar reasons, large differences are also observed in AID of AA among different samples of the same feedstuff (Sauer et al., 1991). To better understand the causes of differences in AA digestibility, it is necessary to at the factors causing these differences.

2. *Calculation of ileal digestibility*

2.1 Apparent ileal digestibility coefficients

Apparent ileal digestibility (AID) is calculated as the percentage of AA intake that does not appear in digesta or feces. Apparent ileal digestibility coefficients are dependent upon the AA content in the assay diet. AID coefficients increase curvilinearly as the AA in the assay diet increases (Furuya and Kaji, 1989; Li et al., 1993; Fan et al., 1994)

There is considerable variation in AID among different samples of the same feedstuff. Sauer and Ozimek (1986) stated that there is more variation in AID of cereal grains compared to high protein feedstuffs. Variation due to inherent factors was concluded to be the cause (level of fiber, variety of grain, processing). Moreover, the AID values of ingredients in a ration are not additive.

The word 'apparent' refers to the fact that the coefficients are not corrected for endogenous nitrogen and AA losses. Depending on the amount of endogenous protein in the ileal digesta, the AID is affected to different extent.

There are three methods for measuring AID. These methods are the direct, the difference and the regression method. The direct method is applied to many of the ceral grains, as most of the cereals are very palatable. The difference method is used for low-protein feedsuffs of poor palatability (Mosenthin et al., 2000). Fan and Sauer (1995) concluded that the regression method is the most accurate approach for determining the AID of protein supplements with low inclusion level due to their poor palatability.

2.2 Endogenous losses of amino acids

Endogenous secretions of AA do not directly originate from the diet but from various sources including saliva, pancreatic secretions, sloughed off epithelial cells and mucin (Sauer et al., 2000). Most endogenous protein along with dietary protein is digested and reabsorbed (Fuller, 1991). The majority of the endogenous protein recovered at the distal ileum is composed of sloughed off epithelial cells and mucin as they are partially resistant to enzymatic hydrolysis (Moughan and Schuttert, 1991).

The endogenous secretion and reabsorption influence the amount of protein recovered at the distal ileum, thus affecting the ileal AA digestibility values. The endogenous protein and AA losses (EAL) can be divided into specific losses and non-specific losses (Souffrant, 1991). The non-specific loss is the basal minimum gut loss and it is related to the DM intake of the animal. The non-specific loss is expressed as g per kg dry matter intake and is constant at different dietary AA levels. The specific loss is variable and is related to inherent factors in the feedstuff such as dietary protein, antinutritive factors, and level of fiber. (Mosenthin et al., 2000).

The basal EAL can be assessed by feeding protein-free diets, feeding diets containing protein sources that are assumed to be 100% digestible, and by the regression technique (Boisen and Moughan, 1996; Nyachoti et al., 1997). The specific losses can be assessed only using the isotope dilution technique and the homoarginine method.

2.3. Standardized ileal digestibility

Corrections of AID for specific and non-specific AA acid losses allows for the calculation of 'real' ileal AA digestibility (Low, 1982). When corrections are made for basal endogenous losses only, the standardized ileal digestibility coefficients (SID) are obtained. To calculate SID, the level of basal endogenous loss needs to be measured. The major advantage of SID is that they are additive in mixed diets (Stein et al., 2005). Therefore, using SID increases the precision in diet formulation and they allow feeding the animal closer to its requirement, and thus minimize nitrogen excretion (Rademacher et al., 2001).

2.4 Effects of dietary factors on AA digestibility

Several dietary factors have been reported to influence AA digestibility. Just (1982) reported that the AA digestibility increased with increasing level of crude fat in the diet. Li and Sauer (1994) reported an increase in AID of Lys and Thr with increasing levels of canola oil in the diet. However, Jorgenson and Fernandez (2000) observed no effect of animal fat, palm oil or the combination of the two on AID. Likewise, no effect of added fish oil, rapeseed oil, or coconut oil was observed by these researchers.

The level and type of dietary fiber may affect AID by increasing the endogenous losses. A negative correlation exists between AID and the dietary level of NDF and AID of AA. This is true in both weanling pigs (Li et al., 1994) and growing pigs (Sauer et al., 1991). Mosenthin et al. (1997) evaluated the effect of pectin on apparent ileal and fecal digestibility of AA and on pancreatic secretion in pigs. Dietary pectin (7.5g/100g of cornstarch based diet) significantly reduced the AID for AA. This effect was likely caused by increased endogenous losses because there was no effect of dietary pectin on the secretion of pancreatic proteolytic enzymes.

The addition of antibiotics to a diet may result in an improvement of the digestibility and absorption of AA (Just et al., 1981). The addition of the antibiotics spiramycin or virginiamycin to the diet resulted in reduced production of ammonia and amines. As a result, more lysine was available to the pig and less lysine was converted

into cadaverine in the intestine Dierick (1985). The use of antibiotic in the diet has also been reported to reduce gut microbial activity (Campbell et al., 1982).

Antinutritional factors in the diets also may influence the digestibility of AA. Trypsin inhibitors in legumes and legume products, interfere with the function of trypsin and chymotrypsin (Gabert et al., 1996). Lectins reduce the digestibility of nutrients in the small intestine by binding to the sugars on the surface of the enterocytes (Schulze et al., 1997). Steam treatment, autoclaving, roasting, and extrusion have been shown to inactivate lectins in beans (van der Poel, 1990). Tannins are polyphenolic compounds that bind to proteins and AA (Jansman, 1993). Tannins have also been shown to have adverse effect on AID of CP and AA in pigs (Mitaru et al., 1984).

3. *Effect of level of FI on AA digestibility*

Sauer et al. (1982) fed barley soybean-meal based diets to barrows at 3 levels of FI. The ileal digestibility was assessed with the aid of a ileo-rectal re-entrant cannula. The total tract digestibility was also measured. Levels of FI were 0.84, 1.26, and 1.68 kg per day. The results of this experiment showed no effect of FI on the ileal or total tract digestibility of CP and AA. No decrease in efficiency in digestion and absorption with increasing level of FI were observed. Similar results were reported by Albin et al. (2001) and Haydon et al. (1984).

Peers et al. (1977) fed barley meal at levels of 1 or 3 times the maintenance energy requirement. The apparent digestibility of GE and N did not vary with level of FI. Similar observations were made by Jimmenez (1972).

There is no difference in EAL among growing pigs, lactating sows, and gestating sows given free access to feed (Stein et al., 1999). However, when gestating sows were restricted in their feed intake, an increased EAL was observed. An increase in EAL with decreasing feed intake was also observed by Butts et al. (1993). In an experiment by Hess and Seve (1999) it was concluded that the EAL are proportional to DMI when the FI is higher than 70 g/ $BW^{0.75}$, but not if it is lower. A similar conclusion was reached by Mariscal-Landin et al. (1995).

Based on the above studies, it can be concluded that the level of feed intake does not seem to influence the AID of AA. However, the EAL will decrease with increasing levels of FI. Because SID are calculated by correcting AID for EAL – and because EAL is influenced by FI – it may be speculated that SID are influenced by the level of FI. However, this hypothesis has not yet been tested.

4. The e*ffect of dietary acidifiers on AA digestibility*

Growth promoting effects of organic acids have been reported to be associated with their antibacterial activity and they also have been reported to have physiological effects on the host animal. The hypothesis that organic acids lower the gastric acidity has been used to explain the mode of action of organic acids. Hampson (1994) reported that acidic gastric conditions are detrimental to the survival of the ingested pathogenic bacteria in the stomach. Low gastric pH also aids in the activation of gastric proteases thereby enhancing protein digestion (Cranwell, 1995).

Most studies have been unable to demonstrate that organic acids have any effect in reducing the gastric pH. Canibe et al. (2001) investigated the effect of 1.8% K-diformate

added to the starter diet of weanling pigs. They did not observe significant reduction in gastric or intestinal pH. Scipioni et al. (1978) were able to demonstrate that the addition of fumaric acid or citric acid to piglet feed reduced the pH of stomach contents. The acids were used at the levels of 0.7 and 1%, respectively. Burnell et al. (1988) observed a non-significant reduction in pH of the small intestine in weanling pigs fed a diet with 1.0% sodium citrate. It has been suggested that the supplementation of starter diets with organic acids does not substantially reduce the intestinal pH, therefore decreasing the intestinal pH may not be a primary effect of feeding organic acids (Scipioni et al., 1978; Burnell et al., 1988; Risley et al., 1992).

In contrast to the above reports it was demonstrated by Cole et al. (1968), and Thompson and Lawrence (1981), that dietary organic acids may reduce the gastric pH. Kirchgessner and Roth (1988) reported that the growth promoting effect of K-diformate might be a result of reduction in gastrointestinal pH. Dietary addition of 0.9 or 1.8% k-diformate significantly reduced the pH of the duodenal digesta of weanling pigs by about 0.4 pH units (Mroz and colleagues, unpublished data).

The antibacterial activity of the organic acids is based on the reduction in pH in the diet and the dissociation of acids which is determined by the pK_a value. Undissociated organic acids are lipophilic and can diffuse across the cell membrane. Inside the bacterial cell, the cations and anions that are released by the dissociation of organic acids disrupt their protein synthesis (Roth and Kirchgessner, 1997, 1998; Partenen and Mroz, 1999). Suppression of cell enzymes and nutrient transport systems within the bacterial cell has been reported (Partenen and Mroz, 1999).

Canibe et al. (2001) reported that organic acids whether they come from intestinal bacterial fermentation or are directly supplemented through the feed, have antibacterial effects. They observed a reduction in anaerobic bacteria, lactobacilli, coliforms and yeast. They suggested that this effect might have been due to penetration of undissociated organic acid (K-diformate) rather than reduction of pH in the gastrointestinal tract. Decreased E. coli concentrations have been reported in pigs fed diets supplemented with K-diformate (Overland et al., 2000).

Mroz et al. (2000) reported that the AID for AA is increased if organic acids are included in diets fed to weanling pigs. They suggested that the reduced microbial population in the intestines caused by the acids were responsible for this increase. A reduced microbial count as a result of feeding organic acids has also been reported in broiler chicks (Vogt et al., 1981). In vitro gas production studies have shown that organic acids reduce the microbial fermentation. This effect spares fermentable carbohydrates from microbes to the animal (Piva et al., 2001). Kirchgessner and Roth (1980) studied the influence of fumaric acid on N, DM and energy digestibility. They observed significant improvement in digestibilities. However, this effect was not significant with increasing live weight. Others have not found significant effects of organic acids on protein digestibility (Falkowski and Aherne, 1984; Radecki et al., 1988, Giesting and Easter, 1991; Gabert et al., 1995). Mosenthin et al. (1992) reported that the AID of nitrogen was not affected by propionic acid supplementation, but the AID of Arg, His, Leu, Phe, and Val was improved. Mroz et al. (2000) observed a numeric but not

significant increase in AID of CP and all AA in growing pigs when supplementing diets with formic, fumaric, or n-butyric acid at a level of 300 mmol/kg.

In conclusion, the addition of organic acids to diets for pigs has been shown to reduce the intestinal microbial concentrations, but the gastric and intestinal pH seems to be unaffected by the dietary inclusion of organic acids. The effects of organic acids on the ileal digestibility of AA has not been conclusively elucidated.

5. *Effect of processing on AA digestibility*

The term “feed processing" refers to any treatment to which livestock feed may be subjected. During processing and storage, the nutritional quality of proteins can be improved or reduced by modifications of nitrogen digestibility and/or AA bioavailability. The AA Lys, Trp, Cys, and Met, are most susceptible to changes (Damodaran and Paraf, 1997). Skiba et al. (2001) fed wheat, barley and soybean meal based diets to 40 d old pigs in a mash form, steam pelleted form, mild heat treated form and heavy heat-treated form. Heat treatment reduced ileal digestibility of most nutrients and increased the digestibility in the hindgut. Pelleting increased ileal digestibility of most nutrients and led to the best total tract digestible energy and overall nutritional values. Yang et al. (2001) evaluated the effects of wet feeding of processed diets on nutrient digestibility in young pigs by feeding mash diets, pelleted diets, and expanded crumble diets in a dry form or a wet form. There was no significant difference in nutrient digestibility among treatments. However, pigs fed pelleted diets had increased digestibility compared to pigs fed mash diets.

Heating affects both proteins and carbohydrates, and the presence of moisture tends to increase the effectiveness of heating. Proteins get partially denatured and makes digestion more effective. However, overheating of proteins may lead to a decrease in digestibility because of the formation of Maillard products (Friedman, 1996). Over heated proteins have been shown to have a low AA bioavailability (Batterham et al., 1990). The extent of heat damage will vary depending on the physical composition of the protein concentrate, the heating time, and the pressure and moisture conditions during heating (van Barneveld et al., 1994a).

For the AA that show a significant decrease in digestibility with heat treatment, particularly Lys, the heat modifies the protein structure in such a way that the enzyme hydrolysis associated with the digestion is hindered (Varnish and Carpenter, 1975). Similarly the fact that the ileal digestibility of some AA is significantly increased by the application of heat at 165 ^{0}C indicates that this treatment predisposes the AA for enzyme attack (van Barneveld et al., 1994b). The effect of heating field peas on proximate analysis and total AA content, ileal and fecal digestibilities of AA, and DE content was reported by van Barneveld et al. (1994b). Heat at 110^{0} C significantly increased the ileal digestibilities of Ile, Leu, Phe, His, and Arg Heat but increasing the heat to 165^{0}C, decreased the Lys, Cys, and Arg concentration of peas. The reactive Lys content was also decreased with heat treatment and heat treatment depressed diet DM digestibility and dietary energy digestibility.

De Weck et al. (1987) evaluated the oxidative damage of Trp by hydrogen peroxide. In the presence of excess hydrogen peroxide, the rate of oxidation was faster for free Trp than Trp bound to casein or lactalbumin. The results indicated that protein bound Trp is not very sensitive to oxidation, and therefore, loss of Trp would not be a limiting factor in food processing.

The most common forms of thermal processing conducted in the feed industry are pelleting, roasting, steam flaking, and extrusion/expansion. Benefits of feed pelleting include reduction of dust and improved handling characters. Studies have found an enhancement in digestibility of nutrients in swine as a result of feeding pelleted feed (Maxwell and Carter, 2001). Wondra et al. (1995) also reported that pelleted diets improved nutrient digestibility.

Reducing the particle size also has been reported to increase performance of pigs (Hedde et al., 1985.) An improvement in efficiency of gain for finishing pigs was also observed upon reducing the particle size of sorghum from 1500 to 600µm.

Extrusion leads to shearing and gelatinization of starch, denaturation and shearing of protein, destruction of microorganisms and toxicants and dehydration (Maxwell and Carter, 2001). Extrusion of fibrous and starchy feedstuffs can improve nutrient utilization (Maxwell and Carter2001).

6. *Effect of antimicrobials on AA digestbility*

Antimicrobials are compounds that, at low concentrations, suppress or inhibit the growth of microorganisms. This category of compounds includes the antibiotics (naturally occurring substances produced by yeasts, molds, and other microorganisms)

and the chemotherapeutics (chemically synthesized substances). Some mineral elements have antibiomicrobial properties when included in high concentrations in diets (Chromwell, 2001). Antimicrobials are used in the swine industry at sub therapeutic doses for growth promotion. Antimicrobials are also used at therapeutic levels for the treatment of swine diseases. They exert physiological, nutritional, metabolic, and disease control effects on the host animal. Some examples are enhancement of nutrient absorption and feed intake, energy retention, nitrogen retention, vitamin and trace mineral absorption, and increased plasma concentrations of fatty acid, glucose, and calcium. These effects are thought to be elicited by a reduction in the gut wall diameter, a reduced mucosal cell turnover, and a reduced gut energy loss (Gaskins et al., 2002). Antibiotics have also been found to depress the growth of intestinal bacteria that compete with the host animal for nutrients. Kellog et al. (1964) observed that tetracycline inhibited the growth of lactobacilli and the AA requirement of lactobacilli is about the same as the pig. A shift in intestinal bacteria by an antibiotic may also lead to a reduction in ammonia and amine production and a reduction in VFA production. Improved dietary utilization of protein upon the inclusion of an antibiotic growth promoter in the diets to growing pigs was also reported (Moser et al., 1980).

Improvement in nutrient absorption by antibiotics because of a reduced thickness of the intestinal mucous membrane was suggested by Braude et al. (1955). Braude and Johnson (1953) reported that the feeding of chlortetracycline affected nitrogen and water excretion by pigs. Brody et al. (1954) found that tetracyclins inhibited fatty acid oxidation by the mitochondria in rat liver. In chickens, Anderson et al. (1952)

demonstrated that penicillin increased the number of intestinal coliforms that synthesize nutrients that are essential to the host animal. Catron et al. (1953) reported an increased rate of glucose absorption in pigs if an antimicrobial was included in the diet. It has also been suggested that aureomycin decreases the pH of cecal contents, enhances nitrogen absorption and reduces the metabolic activity of the microflora in rats (Doyle, 2001).

In conclusion, the addition of antimicrobial to diets for growing pigs increases the availability of nutrients to the pig because of a reduction in the microbial utilization of nutrients.

7. *Development of digestive capacity in young pigs*

The changes in diet during early life have an influence on the intestinal morphology, enzyme secretion and nutrient transport (Buddington, 1994). Diets may contain polyamines that do not have a nutritional function but can directly stimulate the growth of the intestine as observed in rats (Butts et al., 1993). Biologically active substances that influence intestinal growth and development (Xu, 1996) have been found in milk and colostrums (Odle et al., 1996), and intestinal cell lines (Cera et al., 1987). Epidermal growth factors and Insulin like growth factors present in milk stimulate intestinal cell proliferation and differentiation (Odle et al., 1996; Xu, 1996).

Post-natal dietary changes also affect the development of the intestinal microflora. In pigs, the anaerobic microflora in the colon are established in the first 2 wk of life (Murray et al., 1987). Diet composition is important in modulating the structure and function of the gastrointestinal tract (Ferraris and Diamond, 1989).

Research has demonstrated that dietary manipulation during suckling or weaning periods have long-lasting and apparently irreversible effects on intestinal transport mechanisms. The critical periods during the programming of irreversible effects in response to environmental conditions has a great significance (Karasov et al., 1985). The critical period which enables irreversible developmental modulation is the weaning period (Pacha, 2000).

Vente Spreeuwenberg et al. (2004) reported that the piglets that received a skim milk powder diet had significantly higher villus height and crypt depth as compared to piglets that received feather meal diet. There also was a positive correlation between ileal digestibility of nutrients and villus architecture. Li et al. (1991) found that pigs fed diets containing SBM had lower VH. Li et al (1991) did not observe any significant difference in VH between pigs fed diets based on soy protein concentrate, moist extruded soy protein concentrate or soy protein isolate.

Zhang et al. (1997) investigated the activities of brush border membrane hydrolases and the rates of sugar and AA uptake by brush border membrane (BBM) vesicles during first 24 h of suckling. Hydrolytic capacity of the entire small intestine increased more for the lactase than for other hydrolases studied. Nutrient transport rate decreased for BBM vesicles for proximal and mid-intestine than for the distal intestine. Transport rate of glucose was reduced than that for galactose and AA.

The effect of age and weaning on the digestive enzymes was studied by Jensen et al. (1997). The activity of trypsin was not affected by weaning whereas that of chymotrypsin and amylase, lipase, colipase, and carboxyl ester hydrolase decreased at

weaning. In contrast the activity of gastric lipase increased. The development of gastric lipase increased before weaning and remained the same post-weaning whereas that of pancreatic lipase, colipase and carboxyl ester hydrolase decreased post-weaning.

Hedemann and Jensen (2004) reported that in stomach tissue of piglets weaned at 4 wk of age, the pepsin activity declined after weaning, but no change in pepsin activity was observed in the gastric contents. Weaning did not affect the activity of gastric lipase. Vente-Spreeuwenberg et al. (2004) reported that the dietary protein source did not affect FI during the initial three days after weaning. During the second wk, the FI of piglets receiving skim milk powder was significantly higher than that of piglets receiving a feather meal diet. The optimal FI to meet protein requirement was attained on d 5 for pigs fed the skim milk powder based diet and on d 7 for pigs fed the feather meal based diet.

It has been demonstrated that the performance of early-weaned pigs is lower with soybean protein diets than with milk protein diets (Walker et al., 1986). Processed soy protein isolates and processed soy protein concentrates (Sohn et al., 1994) gave similar performance to that of dried skim milk based diet. Li et al. (1991) stated that the moist extrusion of soy protein concentrate enhanced the ADG in piglets as compared to SBM, soy protein concentrate and soy protein isolate. Performance of piglets fed moist extruded soy protein concentrate was similar to that of pigs fed dried skim milk-based diet.

Several studies have demonstrated that the protein sources vary in digestibility of AA both at the distal ileum and over the total tract (Sauer et al., 1977; Jorgensen et al.,

1984). Vente Spreeuwenberg (2004) concluded that pigs fed the diet based on skim milk powder had higher AID as compared to pigs fed a feather meal based diet. Sohn et al (1994) reported that the digestibility of N and AA at the distal ileum and over the total tract were greater for pigs fed dried skim milk, isolated soy protein, and soy protein concentrate than the pigs that received soybean meal-based diet.

Walker et al. (1986) indicated that the AID of AA of pigs fed soy protein isolate and soy protein concentrate was better than SBM, however, they were inferior to pigs fed a casein based diet.

Li et al. (1994) observed that in weanling pigs, conventionally processed, SBM might retain antigenic effect as pigs fed SBM in their study had reduced VH. However, there was no difference in VH among pigs fed soy protein concentrate, moist extruded soy protein concentrate or soy protein isolate. Pigs fed the moist extruded soy protein concentrate had the highest ADG among pigs fed soy protein sources.

Makkink et al. (1994) found that the ratio between trypsin and chymotrypsin activity in jejunal chyme was higher for soy fed pigs than for skim milk powder fed pigs. It was concluded that the development of proteases after weaning depends on FI and on dietary protein source.

Moughan et al. (1990) did not observe any difference in apparent absorption of nitrogen and essential AA among the piglets fed liquid milk formulas containing intact bovine milk, hydrolysed bovine milk or isolated soybean protein.

From the above studies it may be concluded that weanling pigs have a lower digestibility of CP and AA than have older pigs. However, this is not the case for milk proteins.

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CHAPTER 3

Effect of Carbadox on the morphology of Porcine small intestine

ABSTRACT: The objective of the current experiment was to assess the effect of the antibiotic carbadox on the intestinal morphology. Twenty weanling piglets (DH X LYD) were used in the experiment. Pigs were weaned at 21 d of age and allotted to one of the two treatment groups. A phase 1 diet was fed to appetite to the pigs during the entire experimental period. Pigs on treatment group 1 received this diet without any in-feed antibiotic (AB-). Pigs on treatment group 2 received the diet with an antibiotic (carbadox) included at 50 ppm (AB+). Small intestinal morphology and enterocyte mitotic index (MI) were assessed on the d of weaning (d-0) on an additional five pigs and on d-5 and d-10 of the experiment. Samples taken from the pigs included intestinal tissue obtained from 33%, 66%, and 100% of the length of the small intestine measured from the pyloric sphincter. Computerized morphometry and enterocyte MI was performed on the intestinal sections. Results were analyzed using a three-way factorial analysis. On d-0, the mean villus height (VH) was higher ($P < 0.05$) than on d-5 and d-10 regardless of the diet being fed post-weaning. Regardless of the site of sampling, there was no effect of diet on VH. Within each diet, there was no difference between mean VH at site 33% and 66%. However, for both diets, VH at both these sites were higher ($P < 0.05$) than at site 100%. There was no difference for CD values in between sites and between diets. The mean CD values for d-0 were lower as compared to d-5 and d-10 ($P < 0.05$). The

mean CD values were highest for d-10 as compared to d-0 and d-5($P < 0.05$). No effects of time, diet, or intestinal location were found for MI. There was no difference in villus height to crypt depth ratio (VH:CD) between the AB- and AB+ diets ($P < 0.05$). For both diets the VH:CD was highest ($P < 0.05$) for d-0, followed by d-5 and d-10 ($P < 0.05$). Site 100% had lowest VH:CD as compared to sites 33% and site 66% ($P < 0.05$). Overall, the results of this experiment demonstrated that VH decreases after weaning while CD increases.

Key Words: Crypt depth, Mitotic index, Villus height

Introduction

The stress associated with weaning pigs at an age of two to three weeks or younger may result in low feed intake, low weight gain, and diarrhea. This stress may result in small intestinal villus atrophy (Cera et al., 1998). Pluske et al. (1996) demonstrated a significant correlation between post-weaning BW gain and villus height (VH). They observed a positive correlation between preservation of VH and crypt depth (CD) and the rate of weight gain.

In the intestinal epithelium, the new cells formed by mitosis in the crypt cells move into the villus epithelium. Villus height and CD are measures of intestinal mucosal morphology (Grant et al., 1990). Measurements of VH give estimation of villus enterocyte numbers. Measurements of CD give a general indication of maturation of enterocytes on the villi. Increased CD indicate increased rate of cell production in the

crypts and migration of enterocytes to the villi, thereby leading to the presence of fewer mature enterocytes on the villus being available for absorption (Hampson, 1986).

The mitotic index (MI) is a measurement of intestinal epithelium proliferation, and is calculated as a percentage of crypt enterocytes in mitosis (Grant et al., 1990). Evaluating the MI in crypt gland cells can assess cellular proliferation in the small intestine (Smith and Jones, 1961; Grant et al., 1990).

There is unequivocal evidence that antibiotic feed supplements increase the growth rate of farm animals. The post-weaning lag in piglets is commonly treated with subtherapeutic doses of antibiotics. However, the use of antibiotics in animals and human beings has resulted in the development of resistant bacteria. Recent findings in Europe have indicated that antibiotic resistance can spread from farm animals to humans (Bager et al., 2000). Mecadox® (carbadox) is one of the most popular in-feed antibiotics used in the United States.

Studying the mechanism of action of antibiotics on the gut will provide information needed to find alternative substances that are capable of mimicking the action of antibiotics. It was the objective of the current experiment to study the effect of Mecadox® on the morphology and MI of the small intestine in weanling pigs.

Materials and Methods

2.1. Animals and housing

A total of 25 piglets originating from the mating of Hampshire x Duroc boars to Large White x Landrace x Duroc sows were used in the experiment. Pigs were weaned at 21 d of age. Average initial BW of the pigs was 5:13 ± 0.63 kg. Pigs were housed in an environmentally controlled room at the Animal and Range Sciences complex, SDSU. The temperature was maintained at 30^0C during the first wk and then decreased by 2^0C during the second wk of the experiment.

2. 2. Experimental design and feeding

On d-0, five pigs were used for the assessment of initial intestinal structure. The remaining 20 pigs were allotted to two treatment groups based on sex, BW, and ancestry. There were 5 pigs per pen and 2 pen replications per treatment group.

On d-5, five pigs from each treatment group were sacrificed and sampled in the same way as the pigs that were sacrificed on d-0. The remaining 10 pigs were sacrificed on d-10.

Two experimental diets were used (Table 1). Both diets were formulated to meet all NRC requirements for 5-10 kg pigs. Diet 1 contained no antibiotic (AB-). Diet 2 contained Mecadox® at 50 ppm % (AB+). Pigs received their experimental diets from d 0. Feed and water was provided for ad libitum access to the pigs.

2. 3. Sampling and data recording

Following administration of Telazol® to the pigs (0.3 mL intra muscular), intestinalectomy was performed and then the pigs were euthanised. (Euthanesia 100 mL vial, Schering, 6-9 mg/lb BW, intra cardiac). From the collected intestine, the mesenteric web was cut allowing the intestine to be laid straight. The small intestine was then placed in physiological saline. Ten cm samples were taken from 33% (proximal jejunum), 66% (distal jejunum), and 100% (distal ileum) of the length of the SI. The samples were tied with cotton strings at both ends. Five to ten mL of ice-cold, 10% neutral buffered formalin was injected into each sample for fixation of the tissue.

After a 4 d fixation period, 6 ring shaped sections were cut from each segment and stored in formalin. Each of these samples were then processed for standard paraffin sectioning and stained with hematoxylin and eosin (H&E). All samples were dehydrated with alcohol using a tissue processor (Pluske et al., 1996).

The best of the 6 slides from each intestinal location were identified and pictures of the slides were taken at 2X magnification with the aid of stereomicroscope (Olympus, SZH10) with mounted video camera screen (Fujifilm digital, HC 3002). Five of the tallest well-oriented villi and their five associated crypts were measured with the aid of image analysis software (Image processing Tool Kit CD-ROM, Version 3 (ISBN # 1-928808-00-X) to give VH and CD values for each location.

The mean VH to CD ratio (VH:CD) was computed as the heights of villi per area in the site of the small intestine divided by the depth of crypts for that site.

Haemotoxylene & Eosin stained slides of the intestinal tissues were used to calculate the MI in crypt gland cells. Light microscopy was used to count a total of 500 crypt gland cells. The cells were counted under 10 X magnification. Mitotic cells of the crypt glands were identified by their structural uniqueness. The absence of a nuclear membrane and separation of chromatids was considered to identify the crypt gland cells undergoing mitosis. The mitotic index was calculated as the number of cells in mitosis per 500 crypt gland cells (Kenworthy, 1976).

2. 4. Calculations and statistical analysis

Villus height, CD, MI, and VH:CD from pigs on d-0, d-5, and d-10 were compared using SAS (SAS Inst, Inc. Cary, NC). A three way factorial analysis was conducted with time, diet, and location as main effects using Proc Mixed.

Results

The mean VH (Table 2) values were highest for d-0, as compared to d-5 and d-10 ($P < 0.05$). However, there was no overall difference in mean VH between d-5 and d-10. Likewise, there was no overall difference in VH between the AB- and AB+ diet (Table 2).

There was no interaction between treatment and site. There was no difference between mean VH values for location 33% and 66%. However, both these sites were higher ($P < 0.05$) as compared to site 100%. Site 33% had the highest mean VH and site 100% had the lowest mean VH ($P < 0.05$). .

There was no interaction between treatment and site for CD values. There was no difference between sites. There was no difference between AB- and AB+ diet. The CD values for d-0 were lowest as compared to d-5 and d-10 ($P < 0.05$). The mean CD values for d-5 were lower as compared to d-10 ($P < 0.05$). Highest CD values were observed for d-10 as compared to d-0 and d-5 ($P < 0.05$).

For VH:CD, there was no difference between AB- and AB+ diets. There VH:CD values of d-0 were highest as compared to d-5 and d-10 ($P < 0.05$) (Table 6). There was no difference in VH:CD between d-5 and d-10. The mean VH:CD values were lowest for d-10 as compared to d-0 and d-5 ($P < 0.05$).

The mean VH:CD for site 33% was highest and different from site 100% ($P < 0.05$) and there was no difference between sites 33% and 66%. Site 100% had the lowest mean VH:CD ($P < 0.05$).

There was no effect of time, site or diet on the MI of crypt gland cells in the small intestine.

Discussion

Analysis of the current data revealed that the mean VH for d-0 were higher than d-5 and d-10 post-weaning. Pluske et al. (1997) reported the presence of bioactive substances (epidermal growth factor, polyamines, insulin-like growth factor-1, L-glutamine) in sow's milk that aid in the intestinal development of young pigs, thereby sustaining the intestinal mucosal integrity. The lack of these substances in the starter diets provided after weaning may contribute to the reduction in VH on d-5 and d-10 post-weaning.

The small intestine of the newly weaned piglet undergoes a reduction in VH and an increase in CD (Pluske et al., 1996). A similar response was observed for the present experiment where the highest VH was observed for d-0, followed by d-5. Grant et al. (1990) reported that VH were higher in piglets on d-7 as compared to d-10. The results of the present experiment are also similar to the findings of van-Beers-Schreurs (1996) who reported that after weaning, villus shortening and crypt deepening in the small intestine of piglets often occurs within a few days.

Results of the present experiment revealed that the mean VH values for the AB+ diet were not higher than those of the group fed AB- diet. This indicates that an in-feed antibiotic does not prevent villi atrophy in early weaned pigs. A similar observation was reported for mice (Comb et al., 1991; Grant et al., 1990).

Spreeuwenberg et al. (2001) reported that effect of diet composition on mucosal integrity is not as important as the effect of low feed intake during first 4-d of post-weaning and in a reparative stage diet effects might be more pronounced. Stress and diminished enteral stimulation compromise the mucosal integrity of the intestine McCracken et al. (1995) and (1999). The present study was conducted for 10-d after weaning, during which time type of diet is not the main cause of villus integrity.

The mean VH values per site were highest in the duodenum, and lowest in the ileum. This finding is in agreement with Rufus (1970). According to Jong (2000), the height of the villi decreases progressively along the length of the small intestine. On the contrary Grant et al. (1990) observed that the villi of duodenum were shorter than villi of jejunum and ileum.

For the present experiment, there was no difference in mean VH:CD ratio between AB+ and AB- diet. This was because of the fact that there was no difference in VH between AB+ and AB– diet. Pluske et al. (1997) reported that a reduction in VH:CD occurs after weaning. The present study showed a reduction in VH:CD with time. Hampson et al. (1986a) suggested that this represented a balance of cell production in the crypts and cell loss from villi that begins on 5-d after weaning and persists for 5-wks.

Grant et al. (1990) reported that CD increased with age which indicated that the size of the proliferative compartment for intestinal epithelial cells increased with age. The present experiment showed an increase in CD with time. Pluske et al. (1997) reported that villus shortening after weaning could occur due to increased rate of cell loss. This in turn leads to increased crypt-cell production that increases the CD.

The MI of crypt gland cells was not affected by time, diet or site. Similarly, Scholten et al. (2002) observed that adding fermented wheat to liquid diets in weaned piglets had no effect on the MI of crypt gland cells. Grant et al. (1990) did not observe the effect of diet on MI in piglets and stated that MI can remain constant with a concomitant increase in CD. MI indicates only a fraction of proliferating cells whereas CD estimates the relative size of the mucosal proliferative compartment. On the contrary, according to Kenworthy (1976), weaned pigs fed diet without antibiotic had increased mitotic indices.

Implications

Antibiotics decrease the incidence of sickness in farm animals, and act as growth promoters. However, emergence of antibiotic resistant bacteria is a public health hazard. Assessment of the action of antibiotics on the gut can aid in the development of alternatives to antibiotic usage in animals. The present study revealed that during the early weaning period an in-feed antibiotic does not aid in enhancing the mucosal integrity of the small intestine. However, future research in this area needs to be done with special emphasis on studies on the factors that help in maintaining the integrity of the gut mucosa.

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Table 1. Ingredient composition of the experimental diet (% as-fed basis)

Diet	1	2
Ingredients, %		
Corn	53.18	53.18
Cornstarch	1.0	0
Whey, dried	10.0	10.0
Soybean meal	23.4	23.4
Fish meal	8.0	8.0
Soybean oil	3.0	3.0
Limestone	0.5	0.5
Dicalciumphosphate	0.2	0.2
L-Lysine HCL	0.1	0.1
L-Threonine	0.02	0.02
Salt	0.4	0.4
Vitamin premix[a]	0.1	0.1
Micromineral premix[b]	0.1	0.1
Mecadox	0	1
Total	100	100

[a]The Vitamin premix provided per kilogram of diet: 10,032.75 IU of vitamin A acetate; 992.25 IU of vitamin D_3 as d-activated animal sterol; 88.25 IU of vitamin E as alphatocopherol acetate; 1.5 mg of vitamin K as menadione dimethypyrimidinol

bisulfate; 0.3975 mg of biotin; 50.75 mg of niacin; 24.75 mg of pantothenic acid as calcium pantohenate; 10 mg of riboflavin; and 0.05 mg of vitamin B_{12}.

[b] The trace mineral premix provided per kilogram of diet: 23 mg of copper as copper sulfate; 110 mg of iron as ferrous sulfate; 0.275 mg of iodine as calcium iodate 23 mg of manganese as manganous oxide; 0.275 mg of selenium as sodium selenite; and 114 mg of zinc as zinc oxide.

Table 2. Effects of diet, time of sampling and intestinal location on villus height (μm)

		AB -				AB +		
Location	Day:	0	5	10	SEM	5	10	SEM
Duodenum		1.81[a c]	0.80[b c]	0.86[b c]	0.04	0.90[b c]	0.99[b c]	0.03
Jejunum		1.71[a c]	0.88[b c]	0.86[b c]	0.05	0.90[b c]	0.83[b c]	0.02
Ileum		1.37[a d]	0.72[b d]	0.82[b d]	0.03	0.88[b d]	0.85[b d]	0.03

[ab] Means with the same superscript are not different over time ($P < 0.05$).

[cd] Means with the same superscript are not different over site ($P < 0.05$).

Table 3. Effects of time of sampling of small intestine on crypt depth (μm)

		AB -				AB +		
Location	Day:	0	5	10	SEM	5	10	SEM
Duodenum		0.63[a]	0.70[b]	0.77[b]	0.01	0.72[a b]	0.88[c]	0.02
Jejunum		0.53[a]	0.78[b]	0.78[b]	0.02	0.65[a b]	0.91[c]	0.02
Ileum		0.64[a]	0.65[b]	0.79[b]	0.01	0.65[a b]	0.95[c]	0.02

[ab] Means with the same superscript are not different over time ($P < 0.05$).

Table 4. Effects of diet, time of sampling, and intestinal location on the villus height to crypt depth ratio

		AB -				AB +		
Location	Day:	0	5	10	SEM	5	10	SEM
Duodenum		2.94[ac]	1.14[bc]	1.13[bc]	0.08	1.27[bc]	1.27[bc]	0.08
Jejunum		3.31[ac]	1.14[bc]	1.11[bc]	0.09	1.41[bc]	0.94[bc]	0.07
Ileum		2.19[ad]	1.11[bd]	1.05[bd]	0.03	1.38[bd]	0.95[bd]	0.06

[abcd]Means with the same superscript are not different. ($P < 0.05$).

Table 5. Overall effect of time, diet and location of small intestine on the mtotic index of crypt gland cells

	Time	Diet	Site
Mean MI	12.60	12.54	12.66
Pr > F	0.3177	0.1011	0.6071

CHAPTER 4

Development of the digestibility of nutrients by weanling pigs

ABSTRACT: The objective of the experiment was to study the development of the apparent ileal digestibility coefficients (AID) for starch, CP, and AA in weanling pigs during the initial 8 wks post-weaning. Twelve suckling pigs (14 d of age) were equipped with a T-cannula in the distal ileum. During the initial 7 d after the surgery, pigs were allowed to continue to nurse the sow, but on d 21, they were weaned and randomly allotted to one of three dietary treatments. The three dietary treatments consisted of diets based on casein, soy isolate, or soybean meal. Lactose, sucrose, and soy oil were included in all diets. Chromic oxide (0.4%) was also included in all diets as an inert marker. Pigs were given free access to their respective diets for eight weeks post-weaning, and samples of ileal digesta were collected during wk 2, 4, 6, and 8. All samples were analyzed for their concentrations of starch, CP and AA, and the AID for these nutrients were calculated. Results of the experiment indicated that there was no change in the digestibility of any of the nutrients as an effect of time post-weaning and there was no time by diet interaction on digestibility. The starch in the casein- and the soyisolate-based diets was almost completely digested and the AID for starch in these two diets were 98.43 and 97.74%, respectively. However, the AID for starch in the soybean meal-based diet was only 88.92% which was lower ($P < 0.05$) than for the other two diets. For CP and all indispensable AA, no differences among diets were observed. It is concluded that the digestibility of starch in newly weaned pigs is not impaired by a

lack of enzyme activity. However, dietary factors such as non-starch polysaccharides may negatively influence starch digestibility. It is also concluded that the AID for AA is lower in newly weaned pigs than in older pigs, and that more than 8 wks post-weaning are needed to improve the digestibility of AA.

Key Words: Amino acids, Digestibility, Phosphorus, Starch, Weanling pigs

Introduction

Enzymes secreted by the porcine digestive system and its accessory glands aid the process of digestion. The salivary glands, stomach, liver, pancreas, and intestine produce these secretions. The synthesis of these digestive fluids is controlled by endocrine, intrinsic, and extrinsic events (Yen, 2001). In very young pigs, enzymes are secreted to digest the nutrients in milk, but enzymes needed for the digestion of nutrients in plant feed stuffs are not secreted (Sohn et al., 1994; Maxwell and Carter, 2001). Therefore, right after weaning, the pigs do not have all the enzymes needed to digest grain-soybean meal diets (Lindemann et al., 1986). However, during the post-weaning period, the pigs go through a transition period and gradually develop the capacity to synthesize the enzymes needed to digest an all-vegetable diet (Makkink et al., 1994; Jensen et al., 1997). The development of enzyme synthesis during this period is highly dependent on the feed intake of the pigs and greater intake results in greater enzyme activities (Makkink et al., 1994). Because of this gradual development of the digestive capacity, it is believed that weanling pigs will gradually develop the capacity to digest nutrients found in plant feed

stuffs. Indeed, it has been reported that the apparent ileal digestibility coefficients (**AID**) of AA are higher in 36-d old pigs than in 27 d-old pigs (Caine et al., 1997). Likewise, Laerke et al. (2003) reported that the digestibility of starch in newly weaned pigs appears to be lower than in older pigs, but a direct comparison of the digestibility of starch in newly weaned pigs and older pigs was not made. It was the objective of the current experiment to study the development of AID of starch, CP, and AA by pigs from weaning and until 12 wk of age.

Materials and Methods

Animals, housing, experimental design, and diets

Twelve nursing piglets (14 d of age) were equipped with a T-cannula in the distal ileum using a procedure adapted from Stein et al. (1998). Following the surgery, pigs were returned to the sow. The pigs were weaned at 21 d of age and housed individually in 1.8- x 0.6m pens for the duration of the experiment in an environmentally controlled room. Room temperature was set at 22^0C but an additional heat source was provided in each pen to create a local temperature of 28-30^0C.

Three diets were prepared using either casein, soy isolate, or soybean meal as the protein source (Table 1 and Table 2). Lactose, sucrose, and soybean oil were included in all three diets. Chromic oxide (0.4%) was also included as an inert marker and vitamins and minerals were included at levels that met or exceeded the estimated requirements for weanling pigs (NRC, 1998). Pigs were randomly allotted to one of the three dietary treatments on the d of weaning with four pigs assigned to each diet. Pigs were given free

access to the diets during the initial eight weeks post weaning. Water was available to the pigs at all times.

Sample collection and chemical analysis

Ileal digesta were collected from the cannulas of all pigs over a 2-d period in wk 2, 4, 6, and 8 post-weaning by attaching a 225 mL-plastic bag to the opened cannulas. Bags were removed every time they were filled with digesta or at least once every 30 min. All samples were stored at -20^{o}C until analyzed. At the end of the experiment, samples were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. All digesta samples were lyophilized and finely ground prior to chemical analysis. Diets and digesta samples were analyzed in duplicate samples for their contents of DM (AOAC, 1998; procedure 4.1.06) and CP (Thiex et al., 2002). Chromium was analyzed in all samples using spectrophotmetry according to Fenton and Fenton (1979). Amino acid concentrations in diets and digesta samples were quantified on a Beckman 6300 Amino Acid Analyzer (Beckman Instruments Corp., Palo Alto, Ca) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Samples were hydrolyzed for 24 h at 110^{o}C with 6 *N* HCL prior to analysis. Methionine and cysteine were determined as methionine sulfone and cysteic acid after cold performic acid oxidation prior to hydrolysis. Tryptophan was determined after alkaline hydrolysis with NAOH for 22 h at 110^{o}C. The concentration of starch was analyzed in the ileal digesta and in diets using a YSI Biochemistry analyzer, Model 2700 (YSI, Yellow Springs, Ohio) according to Xiong et al. (1990).

Calculations and statistical analysis

Apparent ileal digestibility coefficients for AA were calculated using the following equation (Stein et al., 1999):

$$AID = (100-[(AAd/AAf) \times (Crf/Crd)]) \times 100 \quad \textbf{[1]}$$

where AID is the apparent ileal digestibility coefficient of an AA (%), AAd is the AA content in the ileal digesta DM (g/kg), AAf is the AA content in feed DM (g/kg), Crf is the chromium content in the feed DM (g/kg), and Crd is the chromium content in the ileal digesta DM (g/kg). The AID of CP, and starch were calculated using the same equation.

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst, Inc. Cary, NC). The effects of diet and period and the interaction between diet and period were analyzed. An alpha value of 0.05 was used to assess significance between means.

Results and discussion

The results from the experiment are presented in Table 3. There were no interaction between diet and period for any of the variables that were measured. Likewise, there were no effects of period for any of the measurements. Therefore, data were summarized over the four periods and only the main effects of diet are presented. The AID for starch was higher ($P < 0.001$) in the diets based on casein and soy isolate compared to the diet based on soybean meal (98.43 and 97.74 vs. 88.92%). Previously, the AID of starch in corn, sorghum, wheat, barley, and oat groat was found to be close to 100% in growing pigs (Lin et al., 1987). The fact that the pigs fed the casein-based and

the soyisolate-based diets had AID that were close to 100% indicate that young pigs are not limited in their ability to digest starch. This observation also suggests that the secretion of the starch digesting enzymes is sufficient in 4-wk old pigs to ensure complete starch digestion. The majority of the starch in all three diets was corn starch. It is, therefore, likely that the lower digestibility of starch in the soybean meal-based diet compared to the other two diets is caused by factors that are present in the soybean meal. The soybean meal diet had a higher concentration of oligosaccharides and non-starch polysaccharides than had the other two diets. It has been reported that the NDF content of a soybean meal-based diet may increase the rate of passage of digesta, thus, negatively impacting the digestibility of non-fibrous dietary compounds (Martins et al., 2001). The rate of passage was not measured in the present experiment, but the data for starch digestibility give some support to this hypothesis.

There was no difference in the AID of CP among diets. Likewise, for all AA except Glu and Pro, no differences among diets were observed. As mentioned above, there were also no differences among the four periods. This response was somewhat unexpected because previous research has indicated that the AID for AA may increase over time (Caine et al., 1997). For all three feed ingredients that were used in this experiment, the AID were lower compared to what has been previously obtained for older pigs (NRC, 1998). It is, therefore, likely that newly weaned pigs have a lower digestibility of CP and AA compared to older pigs. However, the results from the current experiment indicate that it takes more than eight weeks post-weaning, before the digestibility of AA has reached its peak.

Implication

Results of the current experiment indicate that newly weaned pigs are capable of digesting starch almost completely and that the secretion of the starch digesting enzymes is not limiting starch digestion of weanling pigs. However, it is also indicated that certain dietary factors may reduce the digestibility of starch. In contrast to starch, the digestibility of CP and AA seems to be relatively low in newly weaned pigs compared to older pigs, and there seems to be only a limited improvement in the digestibility during the initial 8 weeks post-weaning.

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Table 1. Ingredient composition of experimental diets (as-fed basis)

Ingredient, %	Diet:	Casein	Soy isolate	Soybean meal
Corn starch		38.12	29.50	12.15
Lactose		25.00	25.00	25.00
Sucrose		10.00	10.00	10.00
Soy isolate		-	30.70	-
Soybean meal, 48%		-	-	48.25
Casein		21.80	-	-
Soybean oil		1.00	1.00	1.00
Limestone		0.78	1.05	1.00
Monocalcium phosphate		2.20	2.10	1.50
Salt		0.50	0.50	0.50
Vitamin premix[a]		0.05	0.05	0.05
Micromineral premix[b]		0.15	0.15	0.15
Chromic oxide		0.40	0.40	0.40
Total		100	100	100

[a] Provided the following quantities of vitamins per kg of complete diet: vitamin A, 10,990 IU as vitamin A acetate; vitamin D_3, 1,648 IU as D-activated animal sterol ; vitamin E, 55 IU as DL-alpha tocopheryl acetate; vitamin K_3, 4.4 mg as menadione dimethylpyrimidinol bisulphite; thiamin, 3.3 mg as thiamine mononitrate; riboflavin, 9.9 mg; pyridoxine, 3.3 mg

as pyridoxine hydrochloride; vitamin B_{12}, 0.044 mg; D-pantothenic acid, 33 mg as calcium pantothenate; niacin, 55 mg; folic acid, 1.1 mg; biotin, 0.17 mg.

[b] Provided the following quantities of minerals per kg of complete diet: Cu, 26 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 0.31 mg as potassium iodate; Mn, 26 mg as manganese sulfate; Se, 0.30 mg as sodium selenite; Zn, 130 mg as zinc oxide.

Table 2. Analyzed nutrient composition of the experimental diets

Item	Diet:	Casein	Soy isolate	Soybean meal
ME, Kcal/kg[a]		3,546	3,506	3,371
Dry Matter, %		92.53	93.35	92.20
Crude protein, %		20.18	27.06	24.08
Calcium[a], %		0.84	0.82	0.82
Phosphorus[a], %		0.70	0.70	0.70
Indispensable AA				
Arginine		0.82	2.28	1.54
Histidine		0.67	0.80	0.58
Isoleucine		1.17	1.48	0.96
Leucine		2.13	2.43	1.66
Lysine		1.78	1.90	1.34
Methionine		0.62	0.40	0.31
Phenylalanine		1.18	1.64	1.10
Threonine		0.91	1.13	0.84
Tryptophan		0.28	0.35	0.35
Valine		1.46	1.51	0.98
Dispensable AA				
Alanine		0.68	1.33	0.94
Aspartic acid		1.56	3.53	2.42

Cysteine	0.09	0.36	0.31
Glutamic acid	4.89	5.77	3.85
Glycine	0.42	1.27	0.91
Proline	2.52	1.60	1.08
Serine	1.08	1.35	0.98
Tyrosine	1.06	1.01	0.71

[a] These values were calculated (NRC, 1998) rather than analyzed.

Table 3. Apparent ileal digestibility coefficients (%) for starch, crude protein, and amino acids by weanling pigs as affected by diet and time after weaning[a]

Item Diet:	Casein	Soy isolate	Soybean meal	SEM	P-value		
					Diet	Period	Diet*Period
Starch	98.43	97.74	88.92	1.50	0.001	0.17	0.09
Crude protein	78.76	76.98	75.94	6.13	0.94	0.79	0.82
Indispensable AA							
Arginine	81.70	89.05	84.31	6.45	0.72	0.86	0.76
Histidine	88.63	84.87	77.94	4.40	0.27	0.84	0.67
Isoleucine	87.08	83.16	73.00	4.90	0.17	0.87	0.66
Leucine	88.07	81.51	72.35	5.40	0.17	0.84	0.61
Lysine	88.70	83.37	73.60	5.15	0.16	0.85	0.61
Methionine	91.95	81.08	77.10	4.80	0.13	0.80	0.45
Phenylalanine	87.89	84.40	74.57	5.20	0.23	0.86	0.67
Threonine	80.99	74.10	65.68	6.25	0.27	0.80	0.62
Tryptophan	85.84	76.74	80.34	5.50	0.52	0.75	0.54
Valine	86.87	78.74	68.79	5.60	0.12	0.82	0.58
Mean, indisp. AA	84.29	80.15	75.43	5.05	0.50	0.44	0.41
Dispensable AA							
Alanine	71.97	77.68	66.11	7.90	0.61	0.87	0.85

Aspartic acid	80.23	85.20	71.32	6.18	0.32	0.94	0.82
Cysteine	37.55	65.63	59.88	15.51	0.43	0.86	0.93
Glutamic acid	90.42	87.76	75.63	3.75	0.04	0.95	0.55
Glycine	59.98	75.83	61.19	9.50	0.45	0.85	0.87
Proline	92.87	75.45	70.87	4.00	0.006	0.64	0.58
Serine	85.40	80.77	72.40	5.02	0.23	0.92	0.60
Tyrosine	89.19	81.33	74.91	5.30	0.18/	0.76	0.51
Mean, dispensable AA	75.98	78.71	69.04	6.80	0.61	0.88	0.80
Mean, all AA	86.76	81.42	72.63	5.00	0.18	0.95	0.70

[a] Data are means of four observations per treatment.

CHAPTER 5

Effect of feed intake on endogenous losses and amino acids and energy digestibility by growing pigs

ABSTRACT: An experiment was conducted to determine the effect of feed intake (FI) on endogenous losses and the digestibility of CP and AA by growing pigs. Six growing barrows (initial BW:70.3 kg) had a T–cannula installed in the distal ileum and were used in a 6 x 6 Latin square design. A soybean meal cornstarch-based diet and a N-free diet were formulated. Chromic oxide (0.25%) was included in both diets as an inert marker. Each diet was provided at three different levels of feed intake (FI). Feed intake level 1 was equal to the estimated energy requirement for maintenance of the pigs, while levels 2 and 3 were two or three times this amount, respectively. Each experimental period lasted 7 d. The initial 4 d of each period was the adaptation period to the experimental diets. On d-5, fecal samples were collected while ileal digesta were collected during two 10-h periods on d-6 and d-7. Between each experimental period, a corn-soybean meal-based diet (16% CP) was fed to all pigs for 7 d. The basal ileal endogenous losses (IAA_{end}) of CP and AA were measured for each level of FI from pigs fed the N-free diet. Likewise, the apparent (AID) and standardized (SID) ileal digestibility coefficients for CP and AA in soybean meal were calculated for each level of FI. The total tract digestibility coefficient of energy in the soybean meal-based diet was calculated as well. The IAA_{end}

of CP and all AA except Pro decreased linearly ($P < 0.05$) as FI increased when expressed as g per kg DMI. However, the total daily IAA_{end} increased as FI increased (linear, $P < 0.05$) for all AA except for Arg, Phe, Thr, Trp, Val, Cys, Gly, and Ser. The AA composition (% of CP) of endogenous protein was not affected by the level of FI, except for Arg, Thr, Pro, and Ser. The AID for CP and all indispensable AA except Lys, Met, Phe, and Thr increased ($P < 0.05$) as FI increased. The SID decreased linearly ($P < 0.05$) for CP and all AA except Arg, Trp, Asp, Pro, and Tyr as FI increased. The total tract digestibility of energy was not influenced by the level of FI. The current results demonstrate that the level of FI significantly influences AID, SID, and IAA_{end} for CP and AA. Therefore, pigs used to measure AA digestibility coefficients and IAA_{end} should be fed at a level that is close to what is used under commercial conditions.

Key words: AA digestibility, Endogenous losses, Feed intake, Pigs.

Introduction

The amount of feed consumed voluntarily by pigs is variable and is affected by many factors (Hyuan et al., 1997). However, the level of feed intake (FI) has been shown to have only minimal or no effect on apparent ileal digestibility coefficients (AID) of CP and AA by growing pigs (Sauer et al., 1982; Haydon et al., 1984; Albin et al., 2001).

Standardized ileal digestibility coefficients (SID) yield more precise estimates of the amount of digestible AA in a mixed diet than do AID (Mosenthin et al., 2000; Jansman et al., 2002). To calculate SID, AID have to be corrected for the basal ileal

endogenous losses (IAA_{end}) of CP and AA (Stein et al., 2001). Endogenous losses of CP and AA are influenced by the level of FI (Butts et al., 1993; Hess and Seve, 1999; Stein et al., 1999b). Therefore, the level of FI is expected to influence SID, but this hypothesis has not been investigated.

Previous experiments investigating the effect of FI on energy digestibility has yielded conflicting results. The DE of barley and of mixed diets has been shown not to vary with the level of FI (Dammers, 1964; Peers et al., 1976). However, Tollet et al. (1961) reported an improvement in DE as FI increased, whereas Morgan et al. (1975) found a significant decrease in DE with increasing level of FI.

The objective of the current experiment was to determine the effect of different levels of FI on IAA_{end} at the distal ileum and on AID and SID of CP and AA in soybean meal by growing pigs. A second objective was to determine the influence of the level of FI on the total tract digestibility of energy in a soybean meal cornstarch-based diet.

Materials and Methods

Animals, housing, and experimental design

Six growing barrows (average initial BW: 70.3 kg) were obtained from the SDSU Swine Research Farm. A T-cannula was installed in the distal ileum of each pig using a procedure adapted from Stein et al. (1998). Following surgery, pigs were allowed to recuperate for 14 d. The pigs were housed individually in 1.2 x 1 m pens for the duration of the experiment in an environmentally controlled room. Room temperature was maintained at 20^0C. A 6 x 6 Latin square design was used with six periods and six

animals representing the rows and the columns, respectively. The experimental protocol was reviewed and approved by the South Dakota State University Animal Care and Use Committee (# 01-A023).

Diets, feeding, and sample collection

Two diets were prepared. Diet 1 was a soybean meal-based diet and diet 2 was a N-free diet (Tables 1 and 2). Chromic oxide (0.25%) was included in both diets as an inert marker. Vitamins and minerals were included at levels that met or exceeded the estimated requirements for growing pigs (NRC, 1998).

Each of the two diets was fed at three different levels of FI. Because FI may be influenced by the ME concentration in the feed, the three levels of FI were defined in relation to the intake of ME. The ME concentrations in the diets were calculated from the expected ME concentrations in the individual feed ingredients (NRC, 1998). Thus, FI level 1 was calculated to be equal to the energy requirement for maintenance (i.e., 106 kcal ME x $kg^{0.75}$; NRC, 1998), whereas levels 2 and 3 were equal to 2 or 3 times this amount, respectively. The daily allotment of feed was divided into two equal meals. Water was available at all times. The pigs were fed the experimental diets at their respective levels of FI for 7 d. The initial 4 d were considered an adaptation period to the diets. On d-5, a fecal sample was collected from each of the three pigs fed the soybean meal-based diet because the energy digestibility was calculated only for the soybean meal-based diet and not for the N-free diet. Digesta were collected at the distal ileum from all pigs on d-6 and d-7 as described previously (Stein et al., 1999a). At the

conclusion of one period, all pigs were provided ad libitum access to a corn soybean meal-based diet (16% CP) for 7 d before the next experimental period was initiated. This procedure was adapted to avoid feeding the pig's energy and protein below their requirements for extended periods of time.

Chemical analysis

At the conclusion of the experiment, digesta and fecal samples were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. All digesta samples were lyophilized and finely ground prior to chemical analysis. Fecal samples were oven dried at 60^0C. Dry matter was determined in diets, digesta, and fecal samples (AOAC, 2000). The concentrations of Kjeldahl N and AA were determined in diets and digesta samples (AOAC, 2000). Amino acids were analyzed on a Chrom-tech HPLC AA analyzer, using ninhydrin for post derivitization and nor-leucine as the internal standard (AOAC, 2000). Samples were hydrolyzed with 6 *N* HCL for 24 h at 110^0C. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight prior to hydrolysis (AOAC, 2000). Tryptophan was determined after samples had been flushed with nitrogen and hydrolyzed with 6 *N* NaOH for 22 h at 110^0C (AOAC, 2000). Amino acid concentrations were not corrected for incomplete recovery resulting from hydrolysis. The chromium concentration of diets, digesta, and fecal samples were determined by spectrophotometry (Fenton and Fenton, 1979). The soybean meal-based diet and fecal samples were analyzed for GE according to AOAC (2000) using a bomb calorimeter (PARR 1563, Moline, IL).

Calculations and statistical analysis

Apparent ileal digestibility coefficients for AA were calculated using equation [1] (Stein et al., 1999a):

$$AID = (100-[(AAd/AAf) \times (Crf/Crd)]) \times 100 \quad \textbf{[1]}$$

where AID is the apparent ileal digestibility coefficient of an AA (%), AAd is the AA concentration in the ileal digesta DM (g/kg), AAf is the AA concentration in feed DM (g/kg), Crf is the chromium concentration in the feed DM (g/kg), and Crd is the chromium concentration in the ileal digesta DM (g/kg). The AID of CP and the total tract digestibility of energy were also calculated using equation [1].

The IAA_{end} of each AA was determined based on the flow obtained after feeding the N-free diet using equation [2] (Stein et al., 1999b):

$$IAA_{end} = [AAd \times (Crf/Crd)] \quad \textbf{[2]}$$

where IAA_{end} is the basal endogenous loss of an AA (g/kg DMI).

The daily flow (g/d) of endogenous CP and AA was calculated by multiplying the IAA_{end} per kg DMI for CP and each AA by the daily DM intake.

By correcting the AID for the endogenous losses of each AA, the SID were calculated for each level of FI using equation [3] (Stein et al., 2001):

$$SID = AID + [(IAA_{end}/AAf) \times 100] \quad \textbf{[3]}$$

where SID is the standardized ileal digestibility coefficient (%). In this calculation, the IAA_{end} used to correct the AID were obtained at FI levels that were identical to those used to determine the AID.

The AA composition of endogenous protein was calculated for each level of FI by expressing each AA as a percentage of total endogenous protein.

The Proc GLM procedure of SAS (SAS Inst., Inc., Cary, NC) was used to evaluate the effect of the level of FI on IAA_{end}, AID, SID, and DE. An analysis of variance was conducted with FI, pig, and period as the main effects. Linear and quadratic effects of FI on IAA_{end}, AID, SID, and DE were determined using Proc GLM with contrast statements.

Results

Apparent ileal digestibility coefficients

The AID for CP, the mean of the indispensable AA, and for all indispensable AA except Arg, Lys, Met, Phe, and Thr increased (quadratic, $P < 0.05$) as FI increased (Table 3). For CP, Ile, Leu, Trp, and Val and for the mean of the indispensable AA, a linear effect ($P < 0.05$) was obtained in addition to the quadratic effect. For Arg, only a linear response was observed ($P < 0.02$) while there was no effect of FI on the AID for Lys, Met, Phe, and Thr.

The AID for Cys and Ser increased linearly as FI increased ($P < 0.04$). Both linear and quadratic responses were observed for Ala, Gly, and the mean of the dispensable AA ($P < 0.03$), but the AID for the remaining dispensable AA were not influenced by FI.

The AID for the mean of all AA showed a linear and a quadratic ($P < 0.03$) response to increased levels of FI.

Endogenous losses of CP and AA

The IAA_{end} measured in g/kg DMI for CP and all AA except Pro decreased linearly ($P < 0.001$) as the FI increased (Table 4). For Pro, there was a tendency for a linear decrease ($P = 0.064$) in IAA_{end} as FI increased. For CP and all AA except Arg, Phe, Thr, Trp, Val, Cys, Gly and Ser, the daily flow to the distal ileum increased (linear, $P < 0.008$ to 0.05) as FI increased (Table 5). In contrast, except for minor changes in the concentration of Arg, Thr, Pro, and Ser, the AA composition of IAA_{end} was not affected by FI (Table 6).

Standardized ileal digestibility coefficients

Except for Arg and Trp, the SID (Table 7) for CP and all indispensable AA and for the mean of the indispensable AA decreased linearly with increasing level of FI ($P =$ 0.003 to 0.035). The SID for the mean of the dispensable AA and for all dispensable AA except Asp, Pro, and Tyr also decreased linearly ($P = 0.002$ to 0.034) with increasing level of FI, as did the SID for the mean of all AA ($P = 0.006$).

Energy digestibility coefficients

The digestibility coefficients for energy in the soybean meal-based diet were 84.8, 87.2, and 86.9% for FI levels 1, 2, and 3, respectively. These values were not significantly different.

Discussion

Apparent ileal digestibility

The AID obtained in the current experiment for pigs fed the highest level of FI are close to values reported for soybean meal by Green and Kiener (1989) and by Fan et al. (1995b). However, the numbers are lower than the AID reported for dehulled soybean meal by Traylor et al. (2001) and by Dilger et al. (2004).

Previous experiments have shown limited or no effects of FI on AID (Sauer et al., 1982; Haydon et al., 1984; Albin et al., 2001). The results of the current experiment are in disagreement with these reports. However, the lowest level of FI used in the above studies was higher than the lowest level used in the present experiment. This may explain why a different response was obtained in the current experiment because the increases in AID are mainly found between low levels of FI.

The reason why AID increase for most AA as FI is increased from a low to a medium level is that IAA_{end} contribute more to the total output of AA at lower levels of FI than at higher levels. Because the ileal output consists of both IAA_{end} and undigested dietary AA, a higher proportion of IAA_{end} will lead to a lower calculated AID. However, the relative influence of IAA_{end} decreases as FI is increased which partly explains why no significant differences in AID are observed at higher levels of FI.

Endogenous losses

The linear decreases in IAA_{end} measured in g per kg DMI for CP and AA that were found in the present experiment confirm previous findings (Butts et al., 1993; Hess

and Seve, 1999; Stein et al., 1999b). In contrast, the daily flow of IAA_{end} increased as FI increased. This is in agreement with James et al. (2002), who observed an increase in the daily loss of all AA with increasing DMI in rats. Similar results were also reported by Butts et al. (1993) and Hess and Seve (1999). Thus, as FI is increased, the IAA_{end} decrease if expressed relative to the DMI of the animals, but they increase if expressed as the daily flow. The reason for this is that one fraction of the basal daily endogenous flow of CP and AA is secreted in response to the DMI of the animals while another fraction is a daily loss secreted regardless of the DMI of the animal (Furuya and Kaji, 1991).

Standardized ileal digestibility

The SID were calculated in the current experiment using the principles previously described for correcting AID for the basal IAA_{end} of CP and AA (Stein et al., 2001; Jansman et al., 2002). The limitation to the use of AID is the assumed lack of additivity of digestibility coefficients for individual feed ingredients in mixed diets (Jansman et al., 2002). The most likely reason for this lack of additivity is that the digesta collected at the distal ileum contains IAA_{end} along with undigested dietary protein which may lead to an underestimation of the AID in feed ingredients with relatively low concentrations of AA (i.e., cereal grains). As the dietary AA levels increase, the contribution of IAA_{end}, as a percentage of total ileal output will decrease which leads to an increase in AID (Fan et al., 1995a). Therefore, calculated AID will change with the dietary concentration of CP and AA. In contrast, SID are independent of the AA concentration in the assay diet, because SID are corrected for IAA_{end}. Thus, by using SID, it has been suggested that

diets can be formulated with greater accuracy (Jansman et al., 2002). However, the results from the current experiment demonstrate that SID are influenced by the level of FI of the animals. To our knowledge, such a finding has not been previously reported.

Because of the design of this experiment, the intake of CP and AA in the soybean meal-based diets increased as the DMI increased. The increased DMI leads to a reduction in basal endogenous losses as illustrated in Table 4. Thus, the DMI *per se* will affect AA digestibility. This effect is responsible for the observed increase in AID at low levels of DMI (Table 3) because the endogenous losses influence AID. If the entire effect of FI on AA digestibility could be explained by the increase in DMI and the resulting reduction in endogenous losses, then the SID should have been constant regardless of the FI of the animals, because IAA_{end} are excluded from the calculations when SID are estimated. However, the SID for CP and all AA except Arg, Trp, Asp, and Pro decreased linearly as FI was increased as illustrated in Table 7. This observation demonstrates that the digestibility of dietary AA is reduced as FI is increased. This effect is caused by the increased AA intake of the animals and is unrelated to the increased DMI. It follows from the above, that the increase in DMI and the increase in CP and AA intake independently affect the AA digestibility in a feed ingredient. This combined effect is included in the calculation of AID, whereas the effects on SID are caused only by the increase in CP and AA intake. The reason why AID tend to plateau at higher levels of FI (i.e., greater than 2 times the energy required for maintenance) is that the reduced IAA_{end} seem to be offset by the reduced digestibility of dietary AA.

A consequence of this observation is that SID are only accurately predicting the digestibility of a diet if the animals that consume this diet have a FI similar to the FI of the animals used to measure the SID. Because many of the SID in the literature were obtained from animals that were restricted in their FI, these values may not be representative of animals that are allowed ad libitum access to feed.

Energy digestibility

In the current experiment, the total tract digestibility of energy was not affected by FI. This is in agreement with previous results (Zivkowic and Bowland, 1963; Peers et al., 1976; Haydon et al., 1984). These results indicate that growing pigs are capable of digesting the energy of a diet with the same efficiency at high levels of FI as at low levels. Thus, substrate availability seems not to influence energy digestibility. Another implication of this finding is that there seems to be no net loss of energy caused by the endogenous loss of CP and AA.

Implications

The level of FI influences IAA_{end}, AID, and SID. While AID are influenced mainly if FI is low, there is a linear effect of FI on SID. As a consequence, feeding the animals close to the voluntary FI during digestibility trials will lead to reduced IAA_{end} per kg DMI and increases the accuracy of the results. Therefore, growing pigs and lactating sows used in studies aimed at determining AID and SID should be fed close to their voluntary FI. However, in gestating sows, restricted feeding is recommended to reflect commercial conditions.

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Table 1. Ingredient composition (%) of the experimental diets (as is-basis)

Ingredient, % Diet:	Soybean meal diet	N-free diet
Soybean meal, 44%	37.5	-
Corn starch	51.75	78.4
Soybean oil	3.0	3.0
Dextrose	5.0	10.0
Solka floc[a]	-	5.0
Limestone	0.5	-
Dicalcium phosphate	1.4	2.75
Chromic oxide	0.25	0.25
Salt	0.4	0.4
Vitamin premix[b]	0.1	0.1
Micromineral premix[c]	0.1	0.1
Total	100	100

[a] Fiber Sales Corp., Urbana, Ohio.

[b] Provided the following quantities of vitamins per kg of complete diet: Vitamin A, 10,032 IU as vitamin A acetate; vitamin D_3, 992 IU as D-activated animal sterol ; vitamin E, 88 IU as DL-alpha tocopheryl acetate; vitamin K_3, 1.52 mg as menadione dimethylpyrimidinol bisulphite; thiamin, 1.5 mg as thiamine mononitrate; riboflavin, 10 mg; Pyridoxine, 4.0 mg

as pyridoxine hydrochloride; vitamin B_{12}, 0.05 mg; D-pantothenic acid, 25 mg as calcium pantothenate; niacin, 60 mg; folic acid, 1.5 mg; biotin, 0.4 mg.

[c] Provided the following quantities of minerals per kg of complete diet: Cu, 25 mg as copper sulfate; Fe, 120 mg as iron sulfate; I, 0.30 mg as potassium iodate; Mn, 25 mg as manganese sulfate; Se, 0.30 mg as sodium selenite; Zn, 125 mg as zinc oxide.

Table 2. Analyzed nutrient composition of the experimental diets (as is-basis)

Item	Diet:	Soybean meal-diet	N-free diet
Dry matter, %		93.85	92.98
Crude protein, %		18.16	0.26
Energy, kcal GE/kg		4,031	3,733
Calcium [a], %		0.60	0.60
Phosphorus [a], %		0.50	0.50
Ash, %		3.37	3.37
NDF, %		6.78	1.6
ADF, %		3.54	2.92
Indispensable AA			
Arginine, %		1.31	0.02
Histidine, %		0.48	-
Isoleucine, %		0.85	0.01
Leucine, %		1.40	0.02
Lysine, %		1.07	0.01
Methionine, %		0.30	0.01
Phenylalanine, %		0.95	0.01
Threonine, %		0.64	0.01
Tryptophan, %		0.20	-

Valine, %	0.87	0.01
Dispensable AA		
Alanine, %	0.81	0.02
Aspartate, %	2.15	0.03
Cysteine, %	0.22	-
Glutamic acid, %	3.21	0.04
Glycine, %	0.78	0.01
Proline, %	0.92	-
Serine, %	1.07	0.01
Tyrosine, %	0.67	0.01

[a] These values were calculated rather than analyzed.

Table 3. Apparent ileal digestibility coefficients (AID) for CP and AA (%) in soybean meal by growing pigs as affected by level of feed intake [a b]

						P-value	
	Level of FI:	Level 1[c]	Level 2[c]	Level 3[c]	SEM	Linear effect	Quadratic effect
Item	DMI, kg/d:	0.78	1.57	2.46	-	-	-
Crude protein		66.6	73.7	71.9	0.66	0.004	0.007
Indispensable AA							
Arginine		86.1	88.5	88.9	0.52	0.014	0.219
Histidine		82.2	86.0	83.8	0.78	0.194	0.048
Isoleucine		73.6	78.4	76.9	0.82	0.039	0.045
Leucine		75.6	80.1	78.4	0.72	0.042	0.035
Lysine		78.5	82.0	79.8	1.13	0.424	0.139
Methionine		78.9	79.6	78.4	1.45	0.805	0.642
Phenylalanine		77.6	81.4	79.8	0.73	0.085	0.055
Threonine		66.5	73.0	71.2	1.58	0.081	0.125
Tryptophan		70.9	76.8	75.8	0.81	0.010	0.035
Valine		69.5	75.5	73.5	0.86	0.019	0.023
Mean, indispensable AA		76.7	80.9	79.4	0.75	0.047	0.049
Dispensable AA							
Alanine		63.1	70.4	67.6	0.97	0.023	0.018

Aspartic acid	79.1	79.9	79.4	1.34	0.846	0.732
Cysteine	64.2	70.5	70.2	0.93	0.008	0.056
Glutamic acid	80.8	82.7	80.7	0.97	0.975	0.206
Glycine	49.9	62.9	61.4	1.06	0.001	0.007
Proline	52.5	54.7	58.2	4.06	0.341	0.919
Serine	73.3	78.4	77.1	0.97	0.040	0.069
Tyrosine	76.4	81.2	79.0	1.00	0.115	0.060
Mean, dispensable AA	72.4	76.1	74.9	0.52	0.018	0.027
Mean, all AA	74.3	78.2	77.0	0.54	0.018	0.023

[a] (100-[(CP or AA in digesta/CP or AA in feed) x (Chromium in feed/Chromium in digesta)]) x 100%.

[b] n = 6.

[c] Level 1 = Body weight x $kg^{0.75}$ x 106 Kcal ME; Level 2 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 2; Level 3 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 3.

Table 4. Flow to the distal ileum (g/kg DMI) of endogenous CP and AA by growing pigs fed a N-free diet as affected by level of feed intake [a b]

						P-value	
	Level of FI:	Level 1 [c]	Level 2 [c]	Level 3 [c]	SEM	Linear effect	Quadratic effect
Item	DMI, kg/d:	0.74	1.46	2.18	-	-	-
Crude protein		48.60	32.45	24.92	2.27	< 0.001	0.182
Indispensable AA							
Arginine		1.53	0.87	0.77	0.12	0.006	0.120
Histidine		0.53	0.34	0.26	0.03	0.002	0.238
Isoleucine		1.20	0.83	0.60	0.08	0.003	0.495
Leucine		1.72	1.19	0.85	0.13	0.005	0.551
Lysine		1.39	0.96	0.71	0.10	0.006	0.502
Methionine		0.41	0.28	0.21	0.03	0.003	0.445
Phenylalanine		1.05	0.74	0.51	0.08	0.006	0.670
Threonine		1.53	0.92	0.68	0.09	0.001	0.178
Tryptophan		0.36	0.23	0.16	0.04	0.011	0.605
Valine		1.48	0.99	0.70	0.10	0.003	0.460
Mean, indispensable AA		1.14	0.74	0.55	0.08	0.003	0.314
Dispensable AA							
Alanine		2.05	1.4	1.03	0.13	0.002	0.382

Aspartic acid	2.57	1.87	1.35	0.24	0.015	0.770
Cysteine	0.53	0.34	0.24	0.03	0.002	0.319
Glutamic acid	3.31	2.38	1.67	0.23	0.004	0.709
Glycine	4.30	2.54	1.97	0.28	0.002	0.140
Proline	10.54	7.36	6.88	1.09	0.064	0.354
Serine	2.05	1.21	0.91	0.12	0.001	0.125
Tyrosine	0.92	0.66	0.46	0.07	0.005	0.695
Mean, dispensable AA	3.29	2.22	1.81	0.22	0.006	0.279
Mean, all AA	2.09	1.39	1.11	0.13	0.003	0.245

[a] [(CP or AA in digesta) x (Chromium in diet/Chromium in digesta)].

[b] n = 6.

[c] Level 1 = Body weight x $kg^{0.75}$ x 106 Kcal ME; Level 2 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 2; Level 3 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 3.

Table 5. Daily flow to the distal ileum (g) of endogenous CP and AA by growing pigs fed a N-free diet as affected by level of feed intake [a b]

						P-value	
	Level of FI:	Level 1 [c]	Level 2 [c]	Level 3 [c]	SEM	Linear effect	Quadratic effect
Item	DMI, kg/d:	0.74	1.46	2.18	-	-	-
Crude protein		34.73	45.55	49.28	2.45	0.008	0.289
Indispensable AA							
Arginine		1.10	1.24	1.50	0.11	0.056	0.642
Histidine		0.38	0.49	0.52	0.04	0.050	0.515
Isoleucine		0.85	1.19	1.20	0.08	0.027	0.178
Leucine		1.21	1.67	1.69	0.13	0.043	0.204
Lysine		0.98	1.36	1.42	0.11	0.037	0.301
Methionine		0.29	0.41	0.41	0.02	0.012	0.113
Phenylalanine		0.74	1.04	1.03	0.09	0.068	0.212
Threonine		1.10	1.32	1.37	0.11	0.155	0.566
Tryptophan		0.26	0.33	0.31	0.04	0.400	0.444
Valine		1.05	1.40	1.40	0.11	0.065	0.221
Mean, indispensable AA		0.81	1.04	1.09	0.08	0.054	0.365
Dispensable AA							
Alanine		1.45	1.95	2.02	0.12	0.022	0.266

Aspartic acid	1.77	2.64	2.72	0.24	0.039	0.238
Cysteine	0.37	0.49	0.48	0.04	0.129	0.278
Glutamic acid	2.34	3.35	3.34	0.23	0.028	0.131
Glycine	3.18	3.52	3.93	0.27	0.101	0.915
Proline	7.83	10.12	13.41	0.95	0.009	0.689
Serine	1.47	1.72	1.83	0.14	0.121	0.716
Tyrosine	0.66	0.93	0.94	0.07	0.039	0.173
Mean, dispensable AA	2.38	3.09	3.58	0.22	0.011	0.704
Mean, all AA	1.51	1.95	2.20	0.13	0.015	0.566

[a] Calculated by multiplying the loss in g/kg DMI by the daily DMI of the animals.

[b] n = 6.

[c] Level 1 = Body weight x $kg^{0.75}$ x 106 Kcal ME; Level 2 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 2; Level 3 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 3.

Table 6. Amino acid composition (% of CP) of endogenous protein in pigs fed an N-free diet at different levels of feed intake[ab]

	Level of FI:	Level 1 [c]	Level 2 [c]	Level 3 [c]	SEM
Item	DMI, kg/d:	0.74	1.46	2.18	-
Crude protein		100	100	100	-
Indispensable AA					
Arginine		3.09 [x]	2.69 [y]	3.03 [xy]	0.10
Histidine		1.10	1.08	1.05	0.04
Isoleucine		2.51	2.62	2.43	0.07
Leucine		3.55	3.69	3.44	0.15
Lysine		2.86	3.00	2.86	0.12
Methionine		0.85	0.89	0.84	0.03
Phenylalanine		2.17	2.29	2.09	0.11
Threonine		3.20 [x]	2.92 [xy]	2.74 [y]	0.10
Tryptophan		0.75	0.72	0.64	0.06
Valine		3.05	3.11	2.82	0.11
Total, indispensable AA		23.1	23.0	21.9	0.75
Dispensable AA					
Alanine		4.17	4.28	4.15	0.11
Aspartic acid		5.11	5.75	5.46	0.38
Cysteine		1.08	1.08	0.99	0.06

Glutamic acid	6.85	7.31	6.71	0.24
Glycine	9.01	7.70	7.87	0.39
Proline	21.34 [x]	21.80 [x]	27.03 [y]	1.47
Serine	4.25 [x]	3.79 [xy]	3.70 [y]	0.13
Tyrosine	1.92	2.06	1.88	0.07
Total, dispensable AA	53.73	53.77	57.80	1.38
Total all AA	76.84	76.77	79.72	1.40

[a] The endogenous loss of each AA was calculated as the percentage of the loss of CP.

[b] n = 6.

[c] Level 1 = Body weight x $kg^{0.75}$ x 106 Kcal ME; Level 2 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 2; Level 3 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 3.

[xy] Means lacking a common superscript letter are different ($P < 0.05$)

Table 7. Standardized ileal digestibility coefficients (SID) for CP and AA (%) in soybean meal by growing pigs as affected by level of feed intake [a b]

						P-value	
	Level of FI:	Level 1[c]	Level 2[c]	Level 3[c]	SEM	Linear effect	Quadratic effect
Item	DMI, kg/d:	0.78	1.57	2.46	-	-	-
Crude protein		91.7	89.1	84.7	1.31	0.003	0.611
Indispensable AA							
Arginine		97.0	94.5	94.3	1.37	0.189	0.533
Histidine		92.5	91.8	88.9	1.10	0.035	0.427
Isoleucine		86.8	86.6	83.5	0.69	0.006	0.125
Leucine		87.1	86.7	84.1	0.60	0.004	0.162
Lysine		90.6	89.6	86.0	1.20	0.017	0.388
Methionine		91.8	88.1	85.0	1.17	0.002	0.871
Phenylalanine		88.0	87.7	84.9	0.60	0.004	0.125
Threonine		88.7	85.8	81.1	2.06	0.019	0.738
Tryptophan		87.0	87.3	82.8	1.78	0.107	0.310
Valine		85.1	84.6	81.0	0.86	0.005	0.176
Mean, indispensable AA		89.9	88.5	85.7	0.85	0.004	0.524
Dispensable AA							
Alanine		86.7	84.1	79.5	1.34	0.002	0.580

Aspartic acid	90.2	88.1	85.3	2.73	0.216	0.939
Cysteine	87.2	84.1	80.7	1.90	0.034	0.953
Glutamic acid	90.4	88.3	85.6	1.28	0.018	0.839
Glycine	101.6	92.2	85.0	3.39	0.004	0.799
Proline	159.2	132.5	127.9	15.05	0.158	0.580
Serine	91.3	88.6	85.2	1.54	0.013	0.850
Tyrosine	89.2	90.0	85.5	1.34	0.071	0.151
Mean, dispensable AA	97.4	92.4	88.8	1.95	0.010	0.800
Mean, all AA	94.0	90.7	87.4	1.42	0.006	0.980

[a] Apparent ileal digestibility + (endogenous loss/intake) x 100%.

[b] n = 6.

[c] Level 1 = Body weight x $kg^{0.75}$ x 106 Kcal ME; Level 2 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 2; Level 3 = Body weight x $kg^{0.75}$ x 106 Kcal ME x 3.

CHAPTER 6

Apparent and standardized ileal digestibility coefficients in three samples of blood cells by growing pigs

ABSTRACT: The objective of the experiment was to evaluate the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) of CP and AA in three different types of spray-dried blood cell products by growing pigs. Five barrows (initial BW: 41.4 ± 3.1 kg) were equipped with a T-cannula in the distal ileum and arranged in a 5 x 5 Latin square design. Diet 1, 2, and 3 contained 8% casein and 8% of each of the three different types of blood cells (BC1, BC2, and BC3). In diet 4, casein was the only source of protein. Diet 5 was an N-free diet. Chromium oxide (0.25%) was used as an inert marker in all diets. The AID for diets 1 to 4 were calculated. By subtracting the portion of AA contributed by casein to diets 1, 2, and 3, the AID for each of the blood cells were also calculated. The endogenous losses (EAL) of AA were calculated from pigs fed the N–free diet. The SID were then calculated by adjusting the AID for endogenous losses. The AID for CP and all the indispensable AA except Ile and Trp were lower for BC2 than for the same AA in BC1 and BC3 ($P < 0.05$). For His, Leu, Lys, Phe, and Val, the AID for BC3 was also higher than for BC1 ($P < 0.05$). For Asp, Ser, and Tyr, BC2 had lower AID than BC1 and BC3 ($P < 0.05$). For Cys, Glu, and the mean of all AA, the AID for BC2 was not different from BC1, but BC2 had lower AID than BC3 ($P < 0.05$). The SID for CP, Arg, and Met were not different between BC1 and

BC3, but BC2 had lower SID for those AA than BC1 and BC3 ($P < 0.05$). For His, Leu, Lys, Phe, and Val, the SID were lower ($P < 0.05$) for BC2 than for BC1, but the SID for these AA were higher in BC3 than in BC2 ($P < 0.05$). For Thr and the mean of the indispensable AA, the SID for BC1 was not different from the SID for BC2 and BC3, but the SID for BC2 was lower than for BC3 ($P < 0.05$). The SID for Ala, Asp, and Ser were not different between BC1 and BC3, but BC2 had lower SID for these AA as compared to BC1 and BC3 ($P < 0.05$). For Glu, the mean of the dispensable AA, and the mean of all AA, BC2 had lower SID than BC3 ($P < 0.05$). The difference in processing of the blood cells is concluded to be the cause for the differences obtained in the AID and SID for these blood cells.

Key words: Amino acids, Blood cells, Digestibility, Processing.

Introduction

In formulating diets for pigs, the ileal digestible amounts of AA rather than total levels of AA should be used (Sauer and de Lange, 1992; Mosenthin et al., 2000). By using such values, the exact needs for dietary AA can be met without over or under supplementation of AA. Therefore, apparent ileal digestibility coefficients (AID) as well as standardized ileal digestibility coefficients (SID) have been determined for a wide range of feed stuffs (NRC, 1998).

Previous research has demonstrated that spray dried blood cells is a good protein source for nursery pigs (Zhang et al. 1998). Limited research has been published on the

digestibility of AA in spray-dried blood cells. NRC (1998) does not report amino acid digestibility values for blood cells.

Spray dried blood cell powder is obtained by spray drying blood cells that have been removed from the unclotted blood via centrifugation (Masters, 1985). Processing by centrifugation to separate plasma from the cells does not jeopardize lys bioavailability of blood cells. Spray dried blood cells and crystalline Lys were found to have similar Lys bioavailibilities (DeRouchy et al.,2002). Beltranena et al. (2003) reported that the spray-dried blood cells have a high concentration of lys. It was the objective of the current experiment to measure AID and SID of the three samples of spray dried blood cells that had been processed using different processing techniques. The hypothesis that different methods for processing the blood cells do not influence AA digestibility was tested.

Materials and methods

Animals, housing, and experimental design

Five growing barrows (Initial BW: 41.4 ± 3.1 kg) were obtained from the SDSU Swine Research Farm and equipped with a T-cannula in the distal ileum using procedures adapted from Stein et al. (1998). Following surgery, pigs were housed individually in 1.2 x 1 m pens for the duration of the experiment in an environmentally controlled room. Room temperature was set at 20^0C. A feeder and a nipple drinker were installed in the pens.

A 5 x 5 Latin square design was used with five periods and five animals representing the rows and the columns, respectively. Each experimental period lasted 7 d. The experiment was approved by the SDSU Animal Care and Use Committee (approval # 01-A 006).

Diets and feeding

The three samples of spray dried blood cells used in the present study differed in the way they were processed. The products BC1 and BC2 contained porcine blood, whereas BC3 contained both porcine and bovine blood. The processing temperature of BC1 and BC2 were similar (73.88^0C), but was different from that of BC3 (240^0C). The BC2 had been treated with hydrogen peroxide prior to spray drying.

The chemical composition of the three types of blood cells and for casein is shown in Table 1. Five diets were prepared (Tables 2 and 3). Diets 1, 2, and 3 contained BC1, BC2, and BC3 respectively, at a level of 8%; in addition, 8% casein was included in each of these diets. In diet 4, casein was the only protein containing ingredient included at 16%. Diet 5 was an N-free diet. Solca floc, a synthetic source of fiber, was included in diet 5 at the level of 4%. Dextrose and soybean oil were included in all diets. Chromium oxide (0.25%) was included in all diets as an inert marker; vitamins and minerals were included at levels that met or exceeded the NRC recommendations for growing pigs (NRC, 1998). Pigs were fed at a level of 3 times the maintenance energy requirement (i.e., 3 x 106 kcal ME/$kg^{0.75}$; NRC, 1998) in two equal meals at 800 and 1800 h. Water was available at all times.

Following surgery, pigs were allowed a two wk recuperation period before the experiment was initiated. During this period, they were fed a standard corn-soybean meal-based diet (16% CP).

Digesta collection

Each experimental period lasted 7 d. The initial 5 d of each period was considered an adaptation period while the remaining 2 d were used for digesta collections in 10 h period as described by Stein et al. (1999a). A plastic bag was attached to the cannula barrel and digesta flowing into the bag was collected. Bags were removed whenever they were filled with digesta or at least once every 30 min and immediately frozen at -20^0C to prevent bacterial degradation of the digesta proteins.

Chemical analysis

At the end of the experiment, samples were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. All digesta samples were freeze-dried and finely ground prior to chemical analysis. Dry matter and Kjeldhal N were analyzed on digesta samples, diets, and the protein containing feed ingredients according to AOAC procedures (AOAC, 2000). Amino acids were analyzed on a Chrom tech HPLC AA analyzer, using ninhydrin for post-column derivitization and nor-leucine as the internal standard (AOAC, 2000). Prior to analysis, samples were flushed with nitrogen and hydrolyzed with 6 *N* HCL for 24 h at 110^0C. Methionine and Cys were determined as met sulfone and cysteic acid after cold performic acid oxidation overnight prior to

hydrolysis. Tryptophan was determined after alkaline hydrolysis for 22 h at 110^0C. The chromium concentration of diets and digesta samples were determined by spectrophotometry as described by Fenton and Fenton (1979).

Calculations and statistical analysis

The AID for AA and CP in diets 1-4 were calculated using equation [1] (Stein et al., 1999a):

$$AID = (100-[AAd/AAf) \times (Crf/Crd)] \times 100 \quad [1]$$

where AID is the apparent ileal digestibility coefficient of an AA (%), AAd is the AA content in the ileal digesta DM, AAf is the AA content in the feed DM, Crf is the chromium content in the feed DM, and Crd is the chromium content in the ileal digesta DM. The AID of CP was also calculated using equation [1].

Results from diet 4 represent the AID for casein. These values were used to calculate the digestibility coefficients for the three blood cells by correcting the values obtained for diets 1, 2, and 3, for the content originating from casein. Thus, apparent ileal digestibility coefficients for the blood cells were calculated using the difference method as suggested by Fan and Sauer (1995). The digestibility of AA in BC 1, 2. and 3 was calculated according to equation [2] (Fan and Sauer, 1995):

$$D_A = (D_D - D_B \times S_B)/S_A \quad [2]$$

Where D_A is the AID of an AA in the blood cells (%). D_D is the AID of an AA in the diet (%). D_B is the AID of an AA in the casein diet.

S_A is the contribution level of an AA from the blood cells to the blood cell containing diet (decimal %). S_B is the contribution level of an AA from casein to the blood cell diet.

The basal endogenous flow to the distal ileum of each AA was determined based on the flow obtained after feeding the N- free diet using equation [3] (Stein et al., 1999b):

EAL = [AAd x (Crf/Crd)] [3]

where EAL is the basal endogenous loss of an AA (g per kg DMI).

By correcting the AID for the EAL of each AA, SID for each diet and for each of the blood cells were calculated using equation [4] (Stein et al., 2001).

SID = [AID + EAL/AAf] [4]

where SID is the standardized ileal digestibility coefficient.

Data were analyzed statistically using the Proc GLM procedure of SAS (SAS Inst. Inc. Cary, NC). An analysis of variance was conducted with pigs, period, and diets as the main effects. Treatment means were separated using the LSMeans statement and the DIFF option of Proc GLM.

Results

Apparent ileal digestibility coefficients for diets

The AID for CP, the mean of all indispensable AA, and all indispensable AA except Ile and Trp were lower for diet 2 ($P < 0.05$) as compared to diets 1, 3, and 4 (Table 4). For Trp, there was no difference in AID between diets 1, 2, and 3, but the AID for diet 2 was lower than for diet 4 ($P < 0.05$). The AID for Ile was lower in diets 1 and

2 than in diets 3 and 4 ($P < 0.05$), but there was no difference between diet 1 and diet 2. There was also no difference in AID for Ile between diets 3 and 4.

For Cys and the mean of all dispensable AA, the AID for diet 2 was lower than for diets 3 and 4 ($P < 0.05$), but there was no difference between diet 1 and diet 2. For Tyr, there was no difference between diets 1, 2, and 3, but diet 4 was higher than the other diets ($P < 0.05$). For Glu, there was no difference between diets 1 and 2, but diet 2 was lower than diets 3 and 4 ($P < 0.05$), and diet 4 was higher than diets 1 and 2 ($P < 0.05$). For Asp and Ser, there was no difference between diets 1, 3, and 4, but diet 2 was lower than diets 1, 3, and 4 ($P < 0.05$). For Ala, there was difference between diets 1, 2, and 3 ($P < 0.05$), and diet 2 had lowest AID. Diet 4 was not different from diet 1, but was different from diets 2 and 3 ($P < 0.05$). For Gly and Pro, there was no difference in AID between diets 1, 2, 3, and 4.

The mean AID for all the AA for diet 2 was lower compared to diets 1, 3, and 4 ($P < 0.05$). The mean AID for diet 4 was also higher than for diet 1 ($P < 0.05$).

Apparent ileal digestibility coefficients for the three types of blood cel1s.

The AID for CP, and all indispensable AA except Ile, Trp, and the mean of the indispensable AA were lower ($P < 0.05$) for BC2 as compared to BC1 and BC3 (Table 5). For His, Leu, Lys, Phe and Val, the AID for BC3 was also higher than for BC1 ($P < 0.05$). There was no difference in the AID for Ile and Trp between BC1, BC2, and BC3. For the mean of all indispensable AA, the AID for BC3 was higher ($P < 0.05$) than for BC2, but BC1 was not different from the other two products.

For the mean of the dispensable AA and for Gly and Pro there was no difference in the AID between BC1, BC2, and BC3. For Ala, the AID for BC2 was lower than for BC1 and BC3 and BC1 was also lower than BC3 ($P < 0.05$). For Asp, Ser, and Tyr, the AID for BC2 was lower than for BC1 and BC3 ($P < 0.05$), but there was no difference between BC1 and BC3. For Cys, Glu, and the mean of all AA, the AID for BC2 was lower ($P < 0.05$) than for BC3, but BC1 was not different from the other two products ($P < 0.05$).

Standardized ileal digestibility coefficients for the experimental diets

The SID for CP, the mean of all indispensable AA, and all indispensable AA except for Ile and Trp were lower for diet 2 ($P < 0.05$) as compared to diets 1, 3, and 4 (Table 6). For Trp, there was no difference in SID between diets 1, 2, 3, and 4. The SID for arg and thr were similar for diets 1, 3, and 4. For His, Leu, Lys, Met, Phe, Val, and the mean of all indispensable AA, there was no difference in SID between diets 3 and 4, but diet 1 was lower than diets 3 and 4 ($P < 0.05$). For Ile, there was no difference between diets 1 and 2, and there was no difference between diets 2, 3 and 4, but the AID for diet 1 was lower than for diets 3 and 4 ($P < 0.05$).

For Ala and Ser, there was no difference between diets 1, 3, and 4, but diet 2 was lower than the other diets ($P < 0.05$) (Table 6). The SID for Asp in diet 2 was lower than for the other diets ($P < 0.05$), and diet 3 was also higher than diets 1 and 4 ($P < 0.05$). The SID for Glu in diet 2 was lower than in diets 3 and 4 ($P < 0.05$) and the SID for diet 4 was also higher than for diet 1 ($P < 0.05$). The SID for Cys, Gly, and Tyr was lower in diet 2 than in diet 4, while diets 1 and 3 were not different from any of the other diets.

For Pro, there was no difference between diets 1, 2, 3, and 4. For the mean of the dispensable AA, there was no difference between diets 1, 3, and 4, but diet 2 was lower than diets 3 and 4 ($P < 0.05$). The SID for the mean of all AA was lower in diet 2 than in the other diets, and diet 1 was also lower ($P < 0.05$) than diet 3 and 4.

Standardized ileal digestibility coefficients for the three blood cells

The SID for CP, Arg, and Met was not different between BC1 and BC3, but BC2 was lower ($P < 0.05$) than BC1 and BC3 (Table 7). For His, Leu, Lys, Phe, and Val, the SID were lower ($P < 0.05$) in BC2 than in BC1 which was lower ($P < 0.05$) than in BC3. For Ile and Trp there was no difference in SID between the three products. For Thr and the mean of the indispensable AA, BC1 was not different from BC2 and BC3, but BC2 was lower than BC3 ($P < 0.05$).

The SID for Ala, Asp, and Ser was not different between BC1 and BC3, but BC2 was lower than BC1 and BC3 ($P < 0.05$). For Cys, Gly, Pro, and Tyr there was no difference between the three blood cells. For Glu, the mean of the dispensable AA, and the mean of all AA, the SID for BC1 was not different from BC2 and BC3, but the SID for BC2 was lower than for BC3 ($P < 0.05$).

Discussion

Blood proteins yield complimentary AA pattern when utilized in animal diets. Plasma proteins and blood cells have high concentration of Lys, Trp, and Thr, but low concentration of Met and Ile (Russel and Weaver, 1996). However, the nutritional value

of a feed protein depends on the distribution of the AA that can be absorbed in a bioavailable form. The bioavilability can be modified during processing and storage (Damodaran and Paraf, 1997).

Spray drying is defined as the transformation of feed from a fluid state into a dried particulate form by spraying the feed into a hot drying medium. The additional processing by centrifugation to separate plasma from the cells does not jeopardize lys bioavailability (DeRouchy et al., 2002). The spray drying process maintains the quality and functionality of protein components and AA digestibility (Russel and Weaver, 1996).

In the present study, the three experimental diets had blood cells included at 8%. The blood cells were of three different types. All three of the blood cells were processed by spray drying. Both BC1 and BC2 contained porcine blood and they were spray dried at 73.88^0C. The BC2 had been treated with hydrogen peroxide at 7% of the condensation of RBC's (approximately 28% solids) prior to spray drying. The BC3 contained 80-90% beef blood and 10-20% pork blood. The BC3 was free flowing, dark reddish-brown granules. The spray drying of BC3 was conducted at 200 atm, with inlet temperature at 240^0C, and the outlet temperature at 90^0C.

The BC2 had a lower concentration of most of the indispensable and dispensable AA compared to BC1 and BC3. However, the gross compositional data alone give little indication of nutritive value of blood meals (Moughan et al., 1999).

In the present study, the blood cells used in diets 1 and 3 were dark brown in color, while the blood cells used in diet 2 had a yellowish color. Moughan et al. (1999) reported that the highest AID was found for a blood meal sample that was noticeably red

in appearance. This was attributed to the fact that utilizing whole blood rather than blood protein coagulation (an oxidative process) will lead to a higher AID. This is in agreement with the finding from the present study, wherein Hence BC2 had undergone the oxidation process with hydrogen peroxide and was not red in appearance. BC2 showed lowered digestibility for CP and AA compared to BC1 and BC3 which were dark brown in color. This is agreement with Moughan et al. (1999) who reported that heating the coagulated blood to 121^0C up to one minute reduced the apparent ileal nitrogen digestibility of blood meal from 91 to 73%.

A combination of factors such as pH, temperature, ionic environment, and the interaction of other food constituents with proteins can cause unpredictable changes in the structural confirmation in proteins (Damodaran and Paraf, 1997).

Moughan et al. (1999) stated that temperature alone might not be the critical factor in determining nitrogen digestibility. It was reported that a combination of heating duration and temperature during processing is important in determining the protein quality of blood meals. In the present study, there was a difference in spray drying temperatures for BC1 and BC3. However, for CP and most AA, the AID and SID of BC3 and BC1 were not different. Hence, the present study indicates that the difference in processing temperature alone may not cause differences in the digestibility of AA in blood cells.

The nutritional quality of the blood meals may be related to protein damage during processing (Kratzer and Green, 1957). Amino acids located adjacent to chemically modified AA cannot be completely liberated from the protein by the intestinal

enzymes and are, therefore, not absorbed. In addition, some reactions lead to cross-linkages between protein chains, which contribute to a reduction in total nitrogen digestibility, and hence, to decreased availability of all other AA (Damodaran and Paraf, 1997). Similarly, in the present study, treatment of BC2 with hydrogen peroxide prior to spray drying might have lowered its nutritional quality.

Sulfur containing AA are sensitive to oxidative reactions. This can happen during processing and storage of proteins in the presence of oxygen through reactions catalyzed by metal ions, via pigments sensitive to light (photo oxidation). In the present study, the AID and SID of Met in BC2 was lower than in BC1 and BC3. However, the AID and SID for Cys of BC2 was not different from BC1 and BC3 samples. The use of hydrogen peroxide during processing, if not well controlled, poses a risk for oxidation. At a low degree of oxidation, the sulfur AA are bioavailable (Anderson et al., 1976).

For Trp, the maximum loss caused by hydrogen peroxide has been found to be less than 25% (Damodaran and Paraf, 1997). This is in agreement with the finding from the present study. No difference was observed in AID and SID of Trp between the three blood cells.

The type of protein in the diet of weanling pigs has an influence on nitrogen digestibility (Peiniau et al., 1996). In a study conducted by Hansen et al. (1993), ADG in pigs was greater in pigs fed plasma from porcine rather than bovine origin. Pigs fed porcine blood had the largest ADFI. In the present study, blood cells BC1 and BC2 were from porcine blood, whereas BC3 contained blood from both porcine and bovine origin. Influence of species on digestibility was not observed in the present experiment, as there

was no significant difference between BC1 and BC3 for the digestibility of most of the AA.

Implications

The present study not only provides the apparent and standardized ileal digestibility values for blood cells, but also reveals that the processing methods affect the digestibility of blood cells. Peroxide treatment may lower the digestibility of amino acids in blood cells. The customers should give importance to qualitative evaluation of the blood cells prior to purchase, as the color of the blood cells reflect their digestibility.

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Table 1. Composition of the blood cell products and casein (as is basis)

Item	Blood cell 1	Blood cell 2	Blood cell 3	Casein
CP, %	96.34	88.80	95.71	92.22
Indispensable AA				
Arginine, %	3.71	3.38	3.80	3.49
Histidine, %	4.64	2.69	7.28	6.09
Isoleucine, %	0.48	4.57	0.42	0.45
Leucine, %	12.72	8.77	12.95	12.58
Lysine, %	7.16	7.05	8.35	8.73
Methionine, %	0.68	2.58	0.66	1.07
Phenylalanine, %	6.27	4.72	6.35	6.87
Threonine, %	2.75	3.81	2.73	3.85
Tryptophan, %	0.30	1.22	1.41	1.69
Valine, %	8.75	5.94	8.63	8.40
Dispensable AA				
Alanine, %	7.58	2.66	7.36	7.52
Aspartic acid, %	11.41	6.29	10.71	9.40
Cysteine, %	0.59	0.34	0.60	0.58
Glutamic acid, %	7.91	20.23	7.68	7.68
Glycine, %	4.47	1.66	4.37	3.93
Proline, %	3.07	9.99	3.15	3.23

Serine, %	3.51	4.91	3.65	3.92
Tyrosine, %	0.48	5.10	1.93	2.40

Table 2. Ingredient composition (%) of experimental diets (as is basis)

Ingredient, %	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Blood cells1	8	-	-	-	-
Blood cells 2	-	8	-	-	-
Blood cells 3	-	-	8	-	-
Casein	8	8	8	16	-
Solca Floc[a]	3	3	3	3	4
Dextrose	5	5	5	5	10
Soybean oil	3	3	3	3	3
Limestone	1.7	1.7	1.7	1.7	0.35
Dicalcium phosphate	2	2	2	2	3.24
Salt	0.4	0.4	0.4	0.4	0.4
Vitamin premix[b]	0.0026	0.0026	0.0026	0.0026	0.0026
Micromineral premix[c]	0.0026	0.0026	0.0026	0.0026	0.0026
Chromium oxide	0.25	0.25	0.25	0.25	0.25
Cornstarch	68.56	68.56	68.56	68.56	78.65
Total	100	100	100	100	100

[a]Fober sales and Development Corp. Urbana, OH.

[b] Vitamin premix provided per kilogram of diet: 260.85 IU of vitamin A acetate; 25.79 IU of vitamin D^3 as d-activated animal sterol; 2.29 IU of vitamin E as alphatocopherol acetate; 0.039 mg of vitamin K as menadione dimethypyrimidinol bisulfate; 0.010 mg of

biotin; 1.13 mg of niacin; 0.64 mg of pantothenic acid; 0.26 mg of riboflavin; and 0.001 mg of vitamin B12.

[C] The trace mineral premix provided per kilogram of diet:0.59 mg of copper as copper sulfate; 2.86 mg of iron as ferrous sulfate; 0.007 mg of iodine as calcium iodate; 0.59 mg of manganese as manganese oxide; 0.007 mg of selenium as sodium selenite; and 2.96 mg of zinc as zinc oxide.

Table 3. Analyzed nutrient composition of the experimental diets (as is basis)

Item	Diet:	Diet 1	Diet 2	Diet 3	Diet 4
Kcal, ME/kg[a]		3,000	3,000	3,000	3,535
Dry Matter, %		90.41	90.46	90.45	89.78
Crude Protein, %		16.53	16.68	16.00	16.52
Calcium, %[a]		0.7	0.7	0.7	0.7
Phosphorous, %[a]		0.57	0.57	0.57	0.61
Indispensable AA					
Arginine, %		0.59	0.55	0.54	0.58
Histidine, %		0.83	0.59	0.69	0.48
Isoleucine, %		0.45	0.41	0.45	0.81
Leucine, %		1.80	1.71	1.69	1.52
Lysine, %		1.31	1.13	1.28	1.27
Methionine, %		0.31	0.26	0.32	0.45
Phenylalanine, %		0.92	0.87	0.92	0.82
Threonine, %		0.55	0.52	0.62	0.64
Tryptophan, %		0.20	0.15	0.20	0.21
Valine, %		1.21	1.15	1.15	1.05
Dispensable AA					
Alanine, %		0.83	0.81	0.80	0.47
Aspartate, %		1.42	1.40	1.25	1.08
Cysteine, %		0.10	0.09	0.10	0.09

Glutamate, %	2.50	2.33	2.45	3.62
Glycine, %	0.50	0.49	0.44	0.30
Proline, %	1.11	1.03	1.10	1.69
Serine, %	0.69	0.64	0.68	0.76
Tyrosine, %	0.52	0.40	0.48	0.74

[a] These numbers were calculated (NRC, 1998) rather than analyzed.

Table 4. Apparent ileal digestibility coefficients (AID) of CP and AA (%) for experimental diets in growing pigs[a b]

Diets	Diet 1	Diet 2	Diet 3	Diet 4	SEM
CP	78.63[y]	69.61[z]	80.29[x y]	85.84[x]	1.99
Indispensable AA					
Arginine	86.30[y]	80.58[z]	88.82[x y]	90.30[x]	1.39
Histidine	91.43[x y]	77.01[z]	94.44[x]	93.70[x y]	0.73
Isoleucine	79.56[y]	80.17[y]	85.41[x]	89.87[x]	1.47
Leucine	89.48[y]	81.71[z]	93.27[x]	93.42[x]	0.84
Lysine	90.40[y]	80.02[z]	93.44[x]	94.11[x]	0.73
Methionine	91.59[y]	88.59[z]	93.92[x]	95.38[x]	0.58
Phenylalanine	90.00[y]	82.24[z]	93.54[x]	93.72[x]	0.75
Threonine	80.30[x]	71.89[y]	84.98[x]	84.91[x]	2.02
Tryptophan	88.83[x y]	85.77[y]	89.70[x y]	91.28[x]	1.39
Valine	86.89[y]	78.50[z]	91.58[x]	90.59[x]	1.09
Mean, indispensable AA	87.48[y]	80.65[z]	90.90[x]	91.73[x]	2.44
Dispensable AA					
Alanine	81.55[y]	70.98[z]	87.20[x]	78.71[y]	1.77
Aspartic acid	84.28[x]	73.82[y]	88.47[x]	86.94[x]	1.54
Cysteine	54.94[x y]	45.29[y]	63.36[x]	64.64[x]	3.99
Glutamic acid	85.12[y z]	83.01[z]	90.17[x y]	92.60[x]	1.67

Glycine	58.78	51.45	63.73	52.39	6.39
Proline	55.77	55.73	63.86	79.01	11.16
Serine	83.67^{x}	76.27^{y}	87.32^{x}	86.89^{x}	1.29
Tyrosine	88.96^{y}	85.74^{y}	89.55^{y}	93.66^{x}	0.81
Mean, dispensable AA	74.13$^{x\ y}$	67.79^{y}	79.21^{x}	79.35^{x}	2.44
Mean, All AA	81.55^{y}	74.93^{z}	85.70$^{x\ y}$	86.23^{x}	1.47

[a](100-[CP or AA in digesta/CP or AA in feed) x (Chromium in feed/Chromium in digesta)]) x 100%.

[b] n = 5.

[x y z]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 5. Apparent ileal digestibility coefficients (AID) of CP and AA (%) for blood cell products in growing pigs [a b]

Item	Blood cell 1	Blood cell 2	Blood cell 3	SEM
CP	71.35[x]	53.20[y]	74.68[x]	3.86
Indispensable AA				
Arginine	82.38[x]	71.06[y]	87.38[x]	2.16
Histidine	90.49[y]	70.15[z]	94.75[x]	1.12
Isoleucine	-20.14	-13.61	42.31	21.07
Leucine	86.57[y]	73.05[z]	93.15[x]	1.63
Lysine	86.87[y]	66.58[z]	92.80[x]	1.63
Methionine	81.31[x]	70.16[y]	89.93[x]	2.85
Phenylalanine	86.97[y]	72.89[z]	93.23[x]	1.49
Threonine	73.78[x]	53.46[y]	85.10[x]	6.01
Tryptophan	86.09	79.58	87.93	2.95
Valine	84.02[y]	69.11[z]	92.34[x]	2.16
Mean, indispensable AA	73.83[xy]	61.24[y]	85.89[x]	3.92
Dispensable AA				
Alanine	82.68[y]	67.89[z]	90.59[x]	2.22
Aspartic acid	82.63[x]	65.67[y]	89.42[x]	3.00
Cysteine	46.91[xy]	29.25[y]	62.30[x]	7.99
Glutamic acid	64.98[xy]	57.22[y]	83.62[x]	7.66
Glycine	61.55	51.05	68.64	9.20

Proline	-20.56	-20.70	14.10	57.17
Serine	79.67[x]	63.06[y]	87.87[x]	3.50
Tyrosine	77.08[x]	65.73[y]	79.14[x]	3.23
Mean, dispensable AA	52.86	47.40	71.96	7.91
Mean, all AA	67.40[xy]	55.09[y]	79.70[x]	4.56

Calculated by subtracting the contribution of casein from the AID of diets 1, 2, and 3 (Table 4).

[b]n = 5.

[x y z] Means within a row lacking a common superscript differ ($P < 0.05$).

Table 6. Standardized ileal digestibility coefficients (SID) of CP and AA (%) for experimental diets in growing pigs[a b]

Diets	Diet 1	Diet 2	Diet 3	Diet 4	SEM
CP	92.06[y]	82.35[z]	94.17[x y]	99.18[x]	1.65
Indispensable AA					
Arginine	96.13[x]	91.14[y]	99.57[x]	100.23[x]	1.43
Histidine	94.10[y]	80.77[z]	97.66[x]	98.30[x]	0.75
Isoleucine	88.44[y]	90.36[x y]	94.69[x]	94.99[x]	1.85
Leucine	93.35[y]	85.79[z]	97.39[x]	97.97[x]	0.91
Lysine	94.58[y]	84.86[z]	97.72[x]	98.39[x]	0.79
Methionine	95.52[y]	93.28[z]	97.72[x]	98.07[x]	0.71
Phenylalanine	94.23[y]	86.72[z]	97.68[x]	98.43[x]	0.85
Threonine	90.97[x]	83.18[y]	94.46[x]	94.02[x]	2.47
Tryptophan	95.81	95.08	96.69	97.88	1.35
Valine	91.66[y]	83.52[z]	96.60[x]	96.05[x]	1.22
Mean, indispensable AA	93.38[y]	87.47[z]	97.02[x]	97.43[x]	1.06
Dispensable AA					
Alanine	90.80[x]	80.46[y]	96.81[x]	94.94[x]	2.01
Aaspartic acid	91.39[y]	81.03[z]	96.54[x]	96.22[x y]	1.66
Cysteine	78.74[x y]	71.75[y]	87.17[x y]	90.90[x]	5.65

Glutamic acid	90.68$^{y z}$	88.99^{z}	95.85$^{x y}$	96.42^{x}	1.77
Glycine	91.12$^{x y}$	84.46^{y}	100.49$^{x y}$	105.90^{x}	5.85
Proline	115.49	120.13	124.15	117.96	10.09
Serine	91.41^{x}	84.62^{y}	95.18^{x}	93.86^{x}	1.45
Tyrosine	96.38$^{x y}$	95.40^{y}	97.59$^{x y}$	98.84^{x}	1.06
Mean, dispensable AA	93.25$^{x y}$	88.35^{y}	99.22^{x}	99.38^{x}	2.27
Mean, all AA	93.32^{y}	87.86^{z}	98.00^{x}	98.30^{x}	1.48

[a]Apparent ileal digestibility of the diet + (endogenous loss/intake) x 100%. Endogenous losses (g/kg DMI) of CP and AA were calculated as the following quantities; CP, 24.53; arg, 0.64; his, 0.24; ile, 0.46; leu, 0.77; lys, 0.60; met, 0.13; phe, 0.43; thr, 0.65; trp, 0.15; val, 0.63; ala, 0.84; asp, 1.11; cys, 0.26; glu, 1.53; gly, 1.78; pro, 7.33; ser, 0.59; tyr, 0.42;

[b]n = 5.

[x y z]Means within a row lacking a common superscript differ *(P < 0.05).*

Table 7. Standardized ileal digestibility coefficient (SID) of CP and AA (%) for blood cell products in growing pigs [a b]

Products	Blood cell 1	Blood cell 2	Blood cell 3	SEM
CP	86.19[x]	67.92[y]	90.02[x]	3.62
Indispensable AA				
Arginine	93.25[x]	82.73[y]	99.26[x]	2.39
Histidine	93.45[y]	74.31[z]	98.31[x]	1.11
Isoleucine	-9.88	-2.35	52.56	21.22
Leucine	90.85[y]	77.56[z]	97.71[x]	1.70
Lysine	91.49[y]	71.93[z]	97.53[x]	1.77
Methionine	85.65[x]	75.34[y]	94.14[x]	2.99
Phenylalanine	91.65[y]	77.84[z]	97.91[x]	1.57
Threonine	85.58[xy]	65.95[y]	95.57[x]	6.42
Tryptophan	93.81	89.88	96.65	2.90
Valine	89.29[y]	74.66[z]	97.90[x]	2.20
Mean, indispensable AA	80.51[xy]	68.78[y]	92.65[x]	4.05
Dispensable AA				
Alanine	92.91[x]	78.38[y]	101.21[x]	2.58
Aspartic acid	90.49[x]	53.64[y]	98.35[x]	3.19
Cysteine	73.24	58.50	88.62	9.71
Glutamic acid	71.13[xy]	63.82[y]	89.90[x]	7.75
Glycine	97.32	87.54	109.29	9.29

Proline	45.40	50.48	80.76	55.71
Serine	88.22[x]	72.28[y]	96.55[x]	3.67
Tyrosine	85.29	76.39	88.03	3.58
Mean, Dispensable AA	72.98[xy]	70.13[y]	94.09[x]	7.23
Mean, all AA	80.51[xy]	69.38[y]	93.29[x]	4.36

[a] Calculated by subtracting the contribution of casein from the SID for diets 1, 2, and 3 (Table 6).

[b] n = 5.

[x y z] Means within a row lacking a common superscript differ *(P < 0.05).*

CHAPTER 7

The Effect of in-feed Aciprol® on the apparent and standardized ileal digestibility in growing pigs.

ABSTRACT: Six growing barrows (Initial BW 59.4±5.4 kg) were used to measure the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) coefficients of a corn soybean meal fish meal-based diet with or without the supplementation of an acidifier. Pigs were fitted with a T-cannula in the distal ileum to gain access to the ileal digesta. Three experimental diets were formulated. Diet 1 was a 22% corn soybean meal fish meal-based diet. Diet 2 was the same diet fortified with 0.5% Aciprol®. Diet 3 was an N-free diet. Chromium oxide was included in all three diets at the level of 0.4% as an indigestible marker. The experimental design was a repeated 3 x 3 Latin square design. Over a 3-wk period, all pigs were fed each of the three experimental diets for 7-d. During each 7-d period, digesta were collected at the distal ileum on d-6 and d-7 for 10 consecutive hrs. Digesta and diet samples were analyzed for CP and AA. The AID for CP, all indispensable AA, and the mean of the indispensable AA were not different between diet 1 and diet 2 except for met and trp. There was an increased AID for trp for diet 2 ($P < 0.05$) as compared to diet 1. The AID for met was higher for diet 1 as compared to diet 2 ($P < 0.05$). The AID for all dispensable AA and the mean of all dispensable AA, except cys were not different between diet 1 and diet 2. The AID for cys was higher for diet 1 as compared to diet 2 ($P < 0.05$). The AID for the mean of all

AA did not differ between diet 1 and diet 2. The SID for CP, and all the indispensable AA, except trp were not different between diet 1 and diet 2. There was an increase in SID for trp for diet 2 ($P < 0.05$) as compared to diet 1. The SID for the mean of all AA was not different between diet 1 and diet 2. The growth promoting effect of acidifiers may not be the result of an enhancement in the AA digestibility. Future research is needed to elucidate the factors that influence the action of acidifiers in the porcine gastrointestinal tract.

Key words: Acidifier, Amino Acids, Digestibility, Pigs.

Introduction

Numerous reports are available on studies that attempted to stabilize the pH of the gastrointestinal tract of pigs by feeding various acidifying agents. Organic acids reduce the gastric pH, thereby reducing bacterial growth, improving pepsin activity, and enhancing energy utilization (Kirchgessner and Roth, 1988). This leads to an improved performance of the pigs. However, the response of pigs to organic acid supplementation depends on the type of diet, the level of acid, the type of acid, and the duration of the feeding period (Gabert and Sauer, 1995).

The use of microencapsulated organic acids is becoming popular. Encapsulated acids are mixtures of acids that are encapsulated by lipids. They are slowly released along the small intestine and the large intestine during digestion (Piva et al., 1997).

Aciprol® consists of a blend of organic and inorganic acids that have been encapsulated using a specific Micropearl® technique. Because of this encapsulation, Aciprol® is believed to be slowly released in the GI-tract of the pig if included in the diet. Only 30% of the acids are released in the stomach while the remaining acids are released in the small intestine and in the colon.

Several investigators have reported improvements in pig performance upon the addition of in-feed acidifiers (Radecki et al., 1988; Giesting et al., 1991). It is believed that the inclusion of encapsulated acids into diets for pigs will improve the digestibility of AA. However, this hypothesis has not yet been tested. It was the objective of this experiment to test the hypothesis that the addition of Aciprol® in diets for pigs will improve AA digestibility prior to the distal ileum.

Materials and methods

Six growing pigs (Initial BW: 59.4±5.4 kg) were used to measure the apparent (AID) and standardized (SID) ileal digestibility coefficients of a corn soybean meal fish meal-based diet with or without the supplementation of Aciprol®. Pigs were equipped with a T-cannula in the distal ileum to gain access to ileal fluids as described by Stein et al. (1998). Three experimental diets were formulated (Tables 1 and 2). Diet 1 was a corn soybean meal fishmeal-based diet with 22% CP. Diet 2 was the same diet fortified with 0.5% Aciprol®, and diet 3 was an N-free diet. Chromium oxide was included in all diets at a level of 0.4% as an indigestible marker, and minerals and vitamins were included at levels that met or exceeded current NRC recommendations (NRC, 1998).

The six pigs were arranged in a repeated 3 x 3 Latin square design. Over a 3-wk period, all pigs were fed each of the three experimental diets for 7-d. The total amount of feed given per d to the pigs was equal to 3 times the maintenance energy requirement (3 x 106 kcal ME/$kg^{0.75}$; NRC, 1998). The initial 5-d of each feeding period was considered an adaptation period while digesta samples were collected from the intestinal cannulas for two twelve h periods on d-6 and 7. All samples were frozen at $–20^0$C immediately after collection to prevent bacterial degradation of digesta proteins.

At the end of the experiment, samples were thawed, mixed within animal and diet, and a sub-sample was taken for chemical analysis. Dry matter and Kjeldahl N were determined in digesta and diet samples (AOAC, 2000). Amino acids were analyzed in diets and digesta on a Chrom-tech HPLC AA analyzer, using ninhydrin for post derivitization and nor-leucine as the internal standard (AOAC, 2000). Prior to analysis, samples were flushed with nitrogen and hydrolyzed with 6 *N* HCL for 24 h at 110^0C. Methionine and cys were determined as met sulfone and cysteic acid after cold performic acid oxidation overnight prior to hydrolysis. Tryptophan was determined after alkaline hydrolysis for 22 h at 110^0C. The chromium content of diets, digesta, and fecal samples were determined by spectrophotometry as described by Fenton and Fenton (1979).

Apparent ileal digestibility coefficients for AA were calculated using equation [1] (Stein et al., 1999a).

$$AID = (100-[(AAd/AAf) \times (Crf/Crd)] \times 100 \quad [1]$$

where AID is the apparent ileal digestibility coefficient of an AA (%), AAd is the AA content in the ileal digesta DM, AAf is the AA content in the feed DM, Crf is the

chromium content in the feed DM, and Crd is the chromium content in the ileal digesta DM. The apparent ileal digestibility of CP was calculated using the same equation.

The basal endogenous flow to the distal ileum of each AA was determined based on the flow obtained after feeding the N-free diet using equation [2] (Stein et al., 1999b):

$$EAL = [AAd \times (Crf/Crd)] \quad [2]$$

where EAL is the basal endogenous loss of an AA (mg per kg DM). The basal endogenous loss for CP was calculated using the same equation.

By correcting the AID for the endogenous loss of each AA, SID were calculated using equation [3] (Stein et al., 2001):

$$SID = [AID + EAL/AAf] \quad [3]$$

where SID is the standardized ileal digestibility coefficient (%).

Data were analyzed statistically using the Proc GLM procedure of SAS (SAS Inst, Inc., Cary, NC). An analysis of variance was conducted with pigs, periods, and diets as main effects. Treatment means were separated using the LSMeans statement and the PDIFF option of Proc GLM.

Results

Apparent ileal digestibility coefficients

The AID for CP and all indispensable AA except met and trp were not different between diet 1 and diet 2 (Table 3). The AID was higher for met in diet 1 as compared to diet 2 ($P < 0.05$). There was an increased AID for trp for diet 2 ($P < 0.05$) as compared

to diet 1. The AID for the mean of the indispensable AA was not different between the two diets.

The AID for all dispensable AA except cys and the mean of all dispensable AA were not different between diet 1 and diet 2. Diet 1 had higher AID for cys as compared to diet 2 ($P < 0.05$). The AID for the mean of all AA did not significantly differ between diet 1 and diet 2.

Standardized ileal digestibility coefficients

The SID for CP and all the indispensable AA except trp were not different between diet 1 and diet 2 (Table 4). There was an increase in the SID for trp in diet 2 ($P < 0.01$) as compared to diet 1. There was no difference in the SID for the mean of the indispensable AA.

The SID for all the dispensable AA, and the mean of all the dispensable AA were not different between diet 1 and diet 2. The mean of all AA showed no significant difference between diet 1 and diet 2.

Discussion

The use of organic acids in their free form can cause palatability problems (Partanen, 2001), damage the stomach and intestinal mucus membrane (Argenzio and Eisemann, 1996), cause bone demineralization (Partanen and Mroz, 1999), and acidic stress inducing resistance to acids in bacteria (Bearson et al., 1997). By protecting the acids, they are supplied to the intestine in a non-dissociated form. Therefore, the acids

get released in the vicinity of the intestinal bacteria (Gautheir, 2002). It has been reported that a combination of organic acids would enhance the effectiveness of acidification, due to their ability to dissociate over a wide range of pH values, thereby maintaining optimum pH through the intestinal tract (Ravindran and Kornegay, 1993).

Aciprol® is a blend of organic and inorganic acids. It contains fumaric acid (20%), citric acid (10%), malic acid (10%), and orthophosphoric acid (10%). Acids in Aciprol® are protected by a lipid based matrix. Microencapsulated organic acids are recommended at a lower rate in the diet than when the organic acids are used in their free form. As these preparations are lipid based, the slow release of these compounds occur during digestion along the small intestine and in the hindgut, allowing organic acids to reach the large intestine (Piva et al., 1997).

Organic acids control the growth of pathogens in the gastrointestinal tract (Russell and Diez-Gonzales, 1988). The spectrum of action of the organic acids depend on the number of carbon atoms they contain and their affinity to lipids (Cherrington et al., 1991). The organic acids are most effective in the stomach as their pK_a is between 3 and 5. However, the organic acids are dissociated in the intestinal tract (Thompson and Hinton, 1997).

The antimicrobial activity of organic acids is believed to be the cause of their positive effect on pigs (Overland et al., 2000; Blank et al., 2001; Canibe et al., 2001). As the microbial population gets reduced, the metabolic need of the microbes is reduced and the availability of dietary energy and nutrients to the host animal is increased, leading to enhanced growth rate and enhanced feed efficiency (Overland et al., 2000). However,

the studies on the antimicrobial properties of organic acids have produced contradictory results.

It has been difficult to demonstrate in vivo that organic acids reduce gastric pH (Partanen and Mroz, 1999). In the intestines, the pancreatic secretions neutralize the effect of organic acids (Argentizo and Southworth, 1975). Harada et al. (1988) reported that short chain fatty acids *per se* might stimulate pancreatic secretions in pigs. This should result in improved ileal AA digestibilities.

In the small intestine, the dissociated organic acids are absorbed across the intestinal epithelia and are eventually metabolized via the citric acid cycle. Pigs could utilize fumaric acid as an energy source via the citric acid cycle with efficiency close to that of glucose (Kirchgessner and Roth, 1982). Thus, organic acids may act as energy sources to help reduce the tissue degradation resulting from protein breakdown and lipolysis.

Aciprol® is believed to release its organic acids slowly along the small intestine and even in the hindgut. In the large intestine, short chain fatty acids produced by microbial fermentation are used as energy sources. This may have a trophic effect in the small intestine; thereby increasing the absorptive area as reported by Sakata (1988), as effects of bacterial metabolism in the large intestine also affects the small intestine.

Amino acid digestibility

The results of the present study are similar to the results by Giesting and Easter (1991), who reported that supplementation of 2% fumaric acid to a corn soybean meal-based diet did not improve ileal digestibility of CP.

Similar to the results of the present study, no difference was observed by Falkowski and Aherne (1984) in apparent total tract digestibility of protein in 4 wk old pigs, upon the addition of 1 or 2% fumaric acid or citric acid to the diet containing grain, dried skim mik, soybean meal, and fish meal. It was concluded that the growth-promoting effect of fumaric acid is not closely correlated to increases in nutrient digestibility.

The effect of supplementation of 2% propionic acid to a soybean meal-based diet for 50 kg barrows was assessed by Mosenthin et al. (1992). No improvement in the AID of the CP was observed by the addition of propionic acid to the diet. However, the AID of the indispensable AA, arg, his, leu, phe, and val were improved by the addition of propionic acid ($P < 0.05$). In the present study an increase in AID of trp was observed, but Aciprol® does not contain propionic acid.

Radecki et al. (1988) added 0, 1.5, or 3% of either fumaric acid or citric acid to corn soybean meal-based diets supplemented with or without antibiotics in 4 wk old weanling pigs. Addition of acids did not have any effect on AID. This finding is in agreement with the present study.

The growth promoting effects of organic acids may be influenced by the buffering capacity of the diet (Ravindran and Kornegay, 1993). Diets with low buffering capacity can act synergestically with acidifiers and lead to increased digestibility. Diets with high

buffering capacity increase the pH leading to alkalinisation and thereby neutralizing acidity (Ravindran and Kornegay, 1993).

Buffering capacity influences the response to organic acids as it compensates for the reduction in gastric pH. A high buffering capacity of the diet decreases the AID of CP and AA (Decuypere et al., 1997; Blank et al., 1999). Soybean meal was found to have a high buffering capacity by Blank et al. (1999). Fishmeal also has a high buffering capacity. In the present study, a corn soybean meal fish meal-based diet was used. It can be inferred that the high buffering capacity of soybean meal and fish meal from the diet in the present study might have neutralized the effect of Aciprol® leading to no difference in digestibility between the control diet and the experimental diet.

Mroz et al. (2000) studied the effect of buffering capacity and organic acids on the AID in barrows. The effect of adding acidogenic Ca benzoate, without and with organic acids (formic, fumaric, and n-butyric acid) on AID were evaluated. The diet with calcium benzoate lowered the buffering capacity of the diet, and enhanced the AID of arg, ile, leu, phe, ala, asp, and tyr by up to 2.4%.

In an experiment by Blank et al. (1999), the inclusion of fumaric acid to a diet with low buffering capacity increased the AID of CP and the majority of AA on d 11 after weaning, but not at d 24 after weaning. It was concluded that acidification could lead to greater responses in early-weaned pigs due to an immature digestive system. This explains the fact that the present study did not show any effect of the acidifier on AID because the animals in the present study were growing pigs.

Standardized ileal digestibility coefficients yield more precise estimates of the amount of digestible AA than AID (Mosenthin et al., 2000). As SID are corrected for EAL, SID are independent of the AA level in the assay diet. Thus, by using SID, it has been suggested that diets can be formulated with greater accuracy (Jansman et al., 2002). The improved SID of trp in acidified diet in the present study needs to be further investigated.

In conclusion, fortifying a corn soybean meal fishmeal-based diet with Aciprol® did not increase neither AID nor SID. The growth promoting effect of acidifiers may not be caused by an improvement in AA digestibility. Future research is needed to assess the functionality of the acidifiers.

Implications

The available data on the effect of organic acids on digestibility in swine show varying results. The present study did not reveal an improvement in digestibility of AA in growing pigs fed a corn soybean meal fish meal-based diet supplemented with Aciprol®. Various factors affect the function of in-feed acidifiers in swine. The concentration of acids, the type of acids, and the buffering capacity of the diet are factors that need to be considered. In addition, the response to acidifiers may vary with the gut environment and the age of the animal. Future research is needed to evaluate the mode of action of in-feed acidifiers in swine.

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Table 1. Ingredient composition (%) of experimental diets (as is basis)

Ingredient Diet:	Control	Aciprol	N-Free
Corn	65.8	65.8	0
Cornstarch	1.0	0.5	78.75
Soybean meal	24.0	24.0	0
Fish meal	5.0	5.0	0
Soy oil	2.0	2.0	3.0
Dextrose	0	0	10
Solka floc[a]	0	0	4
Limestone	0.6	0.6	0
Dicalciumphosphate	0.6	0.6	3.25
Chromic oxide	0.4	0.4	0.4
Salt	0.4	0.4	0.4
Vitamin premix[b]	0.1	0.1	0.1
Micromineral premix[c]	0.1	0.1	0.1
Aciprol®	0	0.5	0
Total	100	100	100

[a]Fober sales and Development Corp. Urbana, OH.

[b] The Vitamin premix provided per kilogram of diet: 10,032.75 IU of vitamin A acetate; 992.25 IU of vitamin D_3 as d-activated animal sterol; 88.25 IU of vitamin E as alphatocopherol acetate; 1.5 mg of vitamin K as menadione dimethypyrimidinol

bisulfate; 0.3975 mg of biotin; 50.75 mg of niacin; 24.75 mg of pantothenic acid as calcium pantothenate; 10 mg of riboflavin; and 0.05 mg of vitamin B_{12}.

[C] The trace mineral premix provided per kilogram of diet: 23 mg of copper as copper sulfate; 110 mg of iron as ferrous sulfate; 0.275 mg of iodine as calcium iodate 23 mg of manganese as manganese oxide; 0.275 mg of selenium as sodium selenite; and 114 mg of zinc as zinc oxide.

Table 2. Analyzed nutrient composition of the experimental diets (as-is basis)

Item Diet:	Control	Aciprol	N-Free
Dry Matter, %	88.29	88.61	89.52
Crude Protein, %	22.15	22.78	0.00
Gross energy Kcal/g[1]	3,390	3,390	3,716
Calcium, %[a]	0.7	0.7	0.7
Phosphorous, %[a]	0.6	0.6	0.6
Indispensable AA			
Arginine, %	1.29	1.27	0.02
Histidine, %	0.55	0.54	0.01
Isoleucine, %	0.87	0.86	0.02
Leucine, %	1.78	1.75	0.04
Lysine, %	1.19	1.16	0.02
Methionine, %	0.39	0.34	0.01
Phenylalanine, %	1.00	0.98	0.02
Threonine, %	0.78	0.76	0.01
Tryptophan, %	0.23	0.28	<0.04
Dispensable AA			
Alanine, %	1.08	1.06	0.03
Aspartate, %	1.98	1.93	0.03

Cysteine, %	0.35	0.34	0.01
Glutamate, %	3.52	3.43	0.08
Glycine, %	0.91	0.90	0.02
Proline, %	1.18	1.10	0.02
Serine, %	0.82	0.78	0.02
Tyrosine, %	0.63	0.64	0.01

[a] These numbers were calculated (NRC, 1998) rather than analyzed.

Table 3. Apparent ileal digestibility (AID) of crude protein and amino acids (%) in experimental diets by growing pigs [a b]

Item Diet:	Control	Aciprol	SEM
CP	71.92	73.05	0.56
Indispensable AA			
Arginine	89.00	88.48	0.69
Histidine	85.22	84.12	0.95
Isoleucine	79.85	78.63	1.21
Leucine	81.44	80.21	1.10
Lysine	82.40	81.18	0.63
Methionine	84.68[x]	82.26[y]	0.75
Phenylalanine	82.18	80.37	1.07
Threonine	73.26	71.42	0.96
Tryptophan	79.15[y]	84.05[x]	1.05
Valine	77.11	75.51	1.25
Mean, indispensable AA	81.43	80.62	0.90
Dispensable AA			
Alanine	76.02	74.46	0.60
Aspartic acid	78.67	77.47	1.02
Cysteine	74.01[x]	72.01[y]	0.93
Glutamic acid	84.66	83.20	0.81

Glycine	69.66	68.42	1.38
Proline	73.86	73.55	2.78
Serine	80.45	78.39	0.67
Tyrosine	81.34	80.67	0.60
Mean, dispensable AA	77.33	76.02	0.72
Mean, all AA	79.61	78.58	0.76

[a] (100-[CP or AA in digesta/CP or AA in feed) x (Chromium in feed/Chromium in digesta)]) x 100%.

[b] n = 6.

[x, y] Means within a row lacking a common superscript differ ($P < 0.05$).

Table 4. Standardized ileal digestibility (SID) of crude protein and amino acids (%) in experimental diets by growing pigs [a b]

Item Diet:	Control	Aciprol	SEM
CP	83.85	84.69	1.58
Indispensable AA			
Arginine	93.76	93.33	0.24
Histidine	90.03	84.61	0.39
Isoleucine	86.12	84.99	1.31
Leucine	86.63	85.51	1.45
Lysine	88.04	86.99	1.81
Methionine	88.65	86.83	0.82
Phenylalnine	87.03	85.61	1.49
Threonine	83.04	81.49	2.05
Tryptophan	86.81[y]	90.36[x]	1.74
Valine	84.64	83.14	1.11
Mean, indispensable AA	87.50	86.73	1.28
Dispensable AA			
Alanine	84.30	82.92	2.22
Aspartic acid	85.42	84.42	1.48
Cysteine	82.83	81.11	1.76
Glutamic acid	89.53	88.22	0.94
Glycine	88.82	87.86	3.67

Proline	120.12	123.35	5.23
Serine	87.49	85.81	1.47
Tyrosine	87.82	87.05	1.47
Mean, dispensable AA	90.79	90.10	1.64
Mean, all AA	88.96	88.23	1.30

[a] Apparent ileal digestibility + (endogenous loss/intake) x 100% Endogenous losses (g/kg DMI) of CP and AA were calculated as the following quantities: CP, 29.93; arg, 0.69; his, 0.30; ile, 0.62; leu, 1.05; lys, 0.76; met, 0.18; phe, 0.58; thr, 1.51; trp, 0.20; val, 0.84; ala, 1.01; asp, 0.05; cys, 0.35; glu, 1.94; gly, 1.97; pro, 6.18; ser, 0.86; tyr, 0.46.

[b] n = 6.

[x, y]Means within a row lacking a common superscript differ ($P < 0.05$).

CHAPTER 8

Summary and general conclusions

The experiments conducted demonstrated that various factors affect the nutrient digestibility in weaner and grower pigs. The study to assess the effect of in-feed antimicrobial on the porcine small intestine revealed that during the early post-weaning period, an in-feed antibiotic does not aid in enhancing the mucosal integrity of the small intestine. However, future research in this area needs to be done with special emphasis on studies on the factors that help in maintaining the integrity of the gut mucosa. Because it is known that antibiotic growth promoters increase the performance of weanling pigs, future work should concentrate on researching other factors (intestinal microflora, intestinal pH, ammonia production, etc.) that may be influenced by antibiotics.

The assessment of effect of age on digestibility of nutrients in weaner pigs revealed that newly weaned pigs seems to have sufficient secretions of starch-digesting enzymes to completely digest the starch in the diet. However, it was also shown that the protein source may affect the digestibility of starch in the diet. This effect is likely caused by the presence of dietary fibers in certain protein sources such as soybean meal. The relatively low apparent ileal digestibility coefficients of amino acids that were obtained in this experiment for both casein, soy protein isolate, and soybean meal, may have been caused by a large endogenous losses caused by low levels of feed intake. The results from this experiment indicate that it is not the secretion of enzymes that limit nutrient

digestibility in newly weaned pigs. However, future experiments are needed to further determine which factors are determine how well newly weaned pigs digest nutrients in the feed.

Upon assessing the effect of level of feed intake on amino acid digestibility, the results of the study indicated that the endogenous losses decrease with an increase in feed intake. Because of the influence of endogenous losses on standardized ileal digestibility coefficients, reduced standardardized ileal digestibility coefficients are observed as the feed intake is increased. Based on these results, it is suggested that feeding pigs on an ad libitum basis during digestibility trials will lead to reduced endogenous losses. This will enhance the accuracy of the results. Reduction in endogenous losses also increases nitrogen retention in the pig thereby increasing the production efficiency.

The experiment conducted to study the effect of processing on the digestibility of amino acids in blood cells not only provides the apparent and standardized ileal digestibility coefficients for blood cells, but also reveals that the processing methods affect the digestibility of blood cells. Peroxide treatment may lower the digestibility of amino acids in blood cells. Buyers of blood cells should give importance to qualitative evaluation of the blood cells prior to purchase, as the color of the blood cells reflect their digestibility.

The assessment of the effect of an in-feed acidifier on ileal digestibility did not reveal an improvement in digestibility of AA in growing pigs fed a corn soybean meal fish meal-based diet supplemented with Aciprol®. Various factors affect the function of in-feed acidifiers in swine. The concentration of acids, the type of acids, and the

buffering capacity of the diet influence the response. In addition, the response to acidifiers may vary with the gut environment and the age of the animal. Future research is needed to evaluate the mode of action of in-feed acidifiers in swine.

Overall, the current experiments suggest that an in-feed antimicrobial is not the sole factor in maintaining the intestinal mucosal integrity in weanling piglets. The feed intake and the type of diet also influence gut health and gut structure in weanling pigs. The in-feed acidifier has been known to enhance growth in pigs; however, the acidifier has no affect on the amino acid digestibility of diets. Hence, the growth promoting action of in-feed acidifiers is due to mechanisms other than enhancement of digestibility. The level of feed intake and the method of processing of feed affect the amino acid digestibility. While feeding pigs, one of the strategies to reduce the endogenous loss is to feed them ad libitum.