Effects of Casein and Protein-free Diets on Endogenous Amino Acid Losses in Pigs**

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ABSTRACT : Quantification of endogenous amino acid loss at the terminal ileum is an essential means for calculation of the true amino acid digestibility of a feedstuff. Since nitrogen appeared in the determined diet or not could shift the results very much, also, none of digestibility markers could be recovered with 100% rate at the terminal ileum, the objectives of the present study were: (1) to determine endogenous amino acid losses when fed either a casein diet or a protein-free diet and (2) to examine the reliability of chromic oxide or acid insoluble ash in the protein-free diet. Six ileal-cannulated pigs (65±1.85 kg BW) with a simple T-cannula in the terminal ileum were used in a replicated 3×3 Latin square designed trial, after allowed a 14 d recuperation period. Each test period ran for 12 days comprised of a 10 d adjustment period and a 2 d collection period. The endogenous AA losses of His, Ile, Lys, Cys, Thr, Val, Trp, Asp, Glu, and Ser from pigs fed the casein diet were significantly higher than those of the protein-free diet ($p<0.05$). No significant difference was found in the amount of endogenous amino acid loss when determined with the different markers in the protein-free diet ($p>0.05$). These data suggest that endogenous amino acid loss could be underestimated when a protein-free diet is used. A direct effect of dietary peptides on the endogenous amino acid loss was found when the casein diet was fed. Our results also indicate that acid insoluble ash can be used as an inert marker as an alternative to chromic oxide when measuring endogenous amino acid loss. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 11 : 1634-1638)***

Key Words : Protein-Free Diet, Casein, Endogenous Amino Acids, Pig, Digestibility Markers

INTRODUCTION

Quantification of endogenous amino acid loss at the terminal ileum is essential in order to calculate the true amino acid digestibility of a feedstuff (Han and Lee, 2000). Traditionally, the endogenous loss of nitrogen and amino acids from the small intestine has been determined after feeding the animal a protein-free diet. However, this method has been criticized for creating a physiologically abnormal state (Low, 1980), which may lead to a decreased rate of whole body protein synthesis (Millward et al., 1976) and thus affect the amount of protein entering the gut.

It is possible that dietary protein or the products of protein digestion have a direct effect on endogenous amino acid excretion. A previous study showed that endogenous amino acid losses from the small intestine were higher under peptide alimentation than under protein-free or synthetic amino acid (SAA) alimentation (de Lange et al., 1990). Therefore, Moughan and Rutherfurd (1990) proposed a new method using enzymically hydrolysed casein for determining ileal amino acid excretion under peptide alimentation.

In the present study, endogenous amino acid losses were determined at the terminal ileum of the pig after feeding either a protein-free diet or a hydrolysed casein diet. In addition, for the protein-free diet, the extent of endogenous amino acid loss was evaluated using two different inert markers (chromic oxide and acid insoluble ash).

MATERIALS AND METHODS

Animals and diets

Six crossbred (Duroc×Landrace Large White) barrows obtained from the Changpin County Pig Farm of Beijing, weighing 40±1.5 kg, were fitted with simple T-cannulas in the terminal ileum (12 to 15 cm anterior to the ileo-cecal junction). The nylon T-cannula, with a threaded 1.8 cm outside diameter tube and curved T-flange 6 cm long, were prepared at the Beijing Agricultural University Machine Shop from nylon rod stock purchased locally. The surgical implantation of the simple T-cannula in the terminal ileum was performed as described by Zhu et al. (1998). During the recuperation period, the pigs were fed a standard corn-soybean meal based diet that met or exceeded their nutrient requirements (NRC, 1998). The experiment was initiated once the barrows reached 65±1.85 kg body weight. The barrows were fed one of three experimental diets (Table 1) according to a replicated 3×3 Latin Square design. Two protein-free diets were formulated with one diet containing 0.5% chromic oxide and the other containing 0.5% acid

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Table 1. Ingredient and chemical composition of experimental diets used to determine ileal endogenous amino acid losses

Ingredients (%, as fed)	Casein diet+chromic oxide	Protein-free diet+chromic oxide	Protein-free diet+Acid insoluble ash
Casein[1]	5.0	-	-
Corn starch[2]	63.6	68.6	68.6
Sucrose	20.0	20.0	20.0
Cellulose	5.0	5.0	5.0
Soybean oil	2.0	2.0	2.0
Limestone	0.3	0.3	0.3
Dicalcium phosphate	1.6	1.6	1.6
Sodium chloride	0.3	0.3	0.3
Premix[3]	1.0	1.0	1.0
Chromium oxide[4]	0.5	0.5	-
Acid insoluble ash	-	-	0.5
Chemical analyses			
Gross energy, MJ/kg	15.2	14.7	14.6
Crude protein[5]	4.44	0.28	0.25
Calcium[5]	0.70	0.72	0.71
Total phosphate[5]	0.61	0.62	0.59

[1] Denoted by Gansu Casein Company with Crude protein 83.20%.

[2] Purchased from Beijing Redstar Starch Company with Gross energy 15.03 MJ/kg, Dry matter 87.30%, Crude protein 0.41%, Calcium 0.074% and Total phosphate 0.0074%.

[3] Premix provided the following per kg of complete diet: vitamin A, 5.512 IU; vitamin D_3, 2.200 IU ; vitamin E, 64 IU; vitamin K_3, 2.2 mg; vitamin B_{12}, 27.6 μg; riboflavin, 5.5 mg; D-pantothenic acid, 13.8 mg; niacin, 30.3 mg; choline chloride, 551 mg; Mn 100 mg; Fe, 100 mg; Zn, 100 mg; Cu, 250 mg; I, 0.3 mg; Se, 0.3 mg.

[4] Purchased from Yixing city Yangxixudu Chemical Company.

[5] Each value represents the mean of chemical analyses conducted in duplicate.

insoluble ash (main component is celite) as a digestibility marker. The third diet contained 5% casein and used chromic oxide as the digestibility marker. Each test period lasted 12 days, consisting of a 10 day adjustment period followed by a 2 day collection of ileal digesta.

Throughout the experiment, the barrows were individually housed in 0.5×1.5 m cast iron metabolic crates equipped with a 0.25 m^3 round bottom feeder located at the front of the crate. The crates were located in an environmentally controlled barn with the temperature maintained at 20±2°C. The barrows were fed at 08:00 h and 20:00 h each day. The amount of feed provided per pig per meal during the experimental period was calculated on the basis of 0.03×body weight at the beginning of the trial and was increased by 350 g between periods. The amount fed was the amount consumed by the pig eating the least during the first 3 days of adjustment phase. Each diet was mixed with water (1:1; w/v) immediately prior to feeding and fresh water was freely available between meals. The barrows typically consumed their ration within 30 min of feeding.

Collection of ileal digesta started one hour after the morning feeding on day 11 of each test period and lasted for 48 h. The cannulas were opened and a soft rubber tube was attached to the barrel of each cannula. The opposite end of the tube was inserted into a plastic 250 ml bottle surrounded by crushed ice. Bottles were replaced at 20 min intervals. Samples were frozen immediately at -20°C and stored for future analyses.

Sample preparation and chemical analyses

Ileal digesta samples were freeze-dried, and then all the samples from the 2 day collection period were pooled and mixed before grinding through a Wiley mill equipped with a 1 mm screen. After grinding, samples were mixed again.

Representative feed samples were analyzed for nitrogen, gross energy, calcium, and total phosphorus using methods of the AOAC (1990). Representative ileal digesta samples were also analyzed for nitrogen using AOAC methods (1990). Chromium content was determined by the flame atomic absorption spectroscopy method after wet ashing using Z-5000 Hitachi Flame Atomic Absorption Spectrometer Analyzer (Kyoto, Japan). Acid insoluble ash content was determined according to the description provided by McCarthy et al. (1974).

Samples of both digesta and diets were subjected to 6M HCl hydrolysis at 110°C for 24 h and acid hydrolysates were subsequently analyzed for amino acid content using a L-8800 Hitachi Automatic Amino Acid Analyzer (Kyoto, Japan). The content of sulfur amino acids (methionine and cystine) was determined using formic acid (9 parts of 88% formic acid plus 1 part 30% hydrogen peroxide) protection before acid hydrolysis. Tryptophan was determined following sodium hydroxide (4.2 N NaOH) hydrolysis (20 h at 110°C) using high-performance liquid chromatography (Shimadzu LC 10A Liquid Chromatograph, Kyoto, Japan).

Statistics

Data were analyzed using GLM model (SPSS 6.0) $Y=\mu+Ai+Bj+Ck+\varepsilon$, where the effects of treatment, period, animal, even interaction between period and animals have been taken into consideration.

RESULTS

A comparison of the ileal endogenous amino acid loss for pigs fed the casein or protein-free diet is presented in Table 2. The ileal endogenous amino acid losses of total indispensable amino acids, total dispensable amino acids and total amino acids from the casein diet were significantly higher than those from the protein-free diets ($p<0.05$). When considered individually, the ileal endogenous amino acid losses of His, Ile, lys, Cys, Thr, Val, Trp, Asp, Glu and Ser from the casein diet were significantly higher than those of the protein-free diet ($p<0.05$). For the two protein-free diets.

Table 2. Ileal endogenous amino acids loss in pigs fed casein and protein-free diets (g/kg DMI)

Item (%)	Casein diet+ Chromic oxide	Protein-free diet+ Chromic oxide	Protein-free diet+ Acid insoluble ash	SEM[1]	P value
Nitrogen	3.11[a]	1.60[b]	2.22[ab]	0.33	0.02
Indispensable AA					
Arginine	0.64	0.31	0.30	0.11	0.07
Histidine	0.39[a]	0.12[b]	0.14[b]	0.04	0.01
Isoleucine	0.51[a]	0.23[b]	0.28[b]	0.05	0.01
Leucine	0.64	0.44	0.51	0.07	0.15
Lysine	0.68[a]	0.31[b]	0.23[b]	0.07	0.01
Methionine	0.13	0.18	0.11	0.04	0.48
Cystine	0.41[a]	0.27[b]	0.26[b]	0.04	0.01
Phenylalanine	0.24	0.26	0.32	0.04	0.31
Tyrosine	0.22	0.15	0.27	0.04	0.15
Threonine	0.91[a]	0.51[b]	0.64[b]	0.08	0.01
Valine	0.86[a]	0.40[b]	0.47[b]	0.07	0.01
Tryptophan	0.15[a]	0.11[b]	0.08[b]	0.01	0.01
Total indispensable AA	5.67[a]	3.28[b]	3.58[b]	0.49	0.01
Dispensable AA					
Alanine	0.68	0.59	0.43	0.13	0.37
Aspartate	1.07[a]	0.63[b]	0.64[b]	0.11	0.03
Glutamate	2.20[a]	0.83[b]	0.94[b]	0.25	0.01
Glycine	2.30	1.02	1.50	0.35	0.06
Proline	1.67	1.69	1.91	0.31	0.40
Serine	1.10[a]	0.44[b]	0.55[b]	0.09	0.01
Total dispensable AA	8.45[a]	4.80[b]	5.07[b]	0.79	0.02
Total AA	14.12[a]	8.08[b]	8.65[b]	1.24	0.01

[1] SEM means standard error of mean.

[ab] Means within a row having different superscripts differ ($p<0.05$).

there was no significant difference in endogenous amino acid loss determined using chromic oxide or acid insoluble ash ($p>0.05$). The total recoveries of chromic oxide and acid insoluble ash were 100.8 and 96.0%, respectively.

DISCUSSION

Traditionally, the endogenous losses of nitrogen and amino acids from the small intestine have been determined after feeding the animal a protein-free diet. However, this method has been criticized for creating a physiologically abnormal state (Low, 1980), which may lead to a decreased rate of whole body protein synthesis (Millward et al., 1976) and thus affect the amount of protein entering the gut (Butts et al., 1993a; Donkoh et al., 1995, 1999). The results of the present experiment show that the ileal endogenous N losses from the casein diet, protein-free diet with chromium, and protein-free diet with acid insoluble ash were 3.11, 1.60 and 2.22 g/kg DMI, respectively, which support that theory.

Animals fed a protein-free diet will mobilize body protein, especially muscle protein, to supply amino acids for vital metabolic functions. Alanine, and especially glutamine, account for more than 50% of the total α-amino acid nitrogen released from muscle tissue (Rodwell, 1985). The tissues of the intestinal tract take up large quantities of glutamine, which can be metabolized to glutamate plus ammonia, citrulline and proline (Rodwell, 1985). In the present experiment, ileal endogenous proline loss in protein-free diets was only numerically higher than that of casein diet ($p>0.05$).

According to Souffrant (1991), the sources of endogenous nitrogen and amino acids secreted into the gastrointestinal tract include saliva, gastric, pancreatic, bile, and small intestinal secretions and sloughed mucosal cells. Of these, small intestinal and pancreatic secretions contribute the most to total endogenous secretions (Chung and Baker, 1992). The principal components of these endogenous secretions are mucoproteins and digestive enzymes, which are rich in Pro, Gly, Glu, Asp, Ser, Ala, Thr and Val. In general, ileal endogenous amino acids present in large quantities are Pro, Gly, Asp, Glu, Thr, Ser, Leu, Ile and Val, whereas those present in small quantities were Met, Try and His. The results of the present experiment are consistent with this research.

The present results (Table 2) provide evidence that the traditional protein-free method for determining ileal endogenous amino acid excretion during growth leads to considerable underestimation of endogenous loss (Butts et al., 1993a,b; de Lange et al., 1989a,b). It appears that there is a direct effect of the products of protein breakdown during digestion on endogenous loss. Total excretion of nitrogen was greater in pigs fed the casein diet than in pigs

fed the protein-free diets (3.11 vs 1.60 and 2.22 g/kg DMI, respectively). Total amino acid nitrogen made up 72.6%, 80.7% and 69.6% of the total nitrogen collected at the ileum of pigs fed casein, or the protein-free diet with chromium or protein-free diets with acid insoluble ash, respectively. The values obtained in the present experiment were close to those obtained by Chung and Baker (1992) where total amino acid nitrogen made up 71.9% and 74.6% of the total nitrogen collected at the ileum of pigs fed protein-free and casein diets, respectively. This suggests that up to 30% of nitrogen in ileal digesta may consist of nucleic acids (bacterial origin), urea and ammonia (Chung and Baker, 1992), an amount similar to that reported by Dierick et al. (1983).

Inert markers are frequently employed in digestibility studies. They provide a means of calculating the digestibility of a nutrient when the complete collection of digesta from a known quantity of feed consumed can not be undertaken. Chromic oxide is the most widespread marker used in studies with pigs (Low, 1982). However, it has been associated with many problems (Jagger et al., 1992), such as variable recovery rates, carcinogenic properties, and oxidizing unsaturated fats. McCarthy et al. (1974) proposed acid insoluble ash as an alternative marker to chromic oxide. Recent work has reported that apparent ileal amino acid digestibility was similar when acid insoluble ash and chromic oxide as digestive markers were used (Zhang et al., 2000). Kavanagh et al. (2001) suggested that acid insoluble ash might be regarded as a reliable technique for the measurement of digestibility of pig diets in metabolism crates after evaluation of chromic oxide, titanium dioxide and acid insoluble ash markers.

In the present experiment, there were no significant differences in the amount of endogenous amino acid loss determined using either acid insoluble ash or chromic oxide used as digestive markers. However, ileal endogenous amino acid losses of the protein-free diet with acid insoluble ash as a marker were numerically higher than those of protein-free diet with chromium as a marker. This may due to the lower recovery rate of acid insoluble ash compared to that of chromic oxide.

IMPLICATIONS

Endogenous amino acids losses could be underestimated when a protein-free diet is fed. A direct effect of dietary peptides on the endogenous amino acids loss was found when a casein diet was fed, supporting that theory. Therefore, the amino acid requirements and availability values obtained from studies using protein-free diets may not correctly determine those values. Also, in digestibility studies, acid insoluble ash can be used as an inert marker as an alternative to chromic oxide.

REFERENCES

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

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

Butts, C. A., P. J. Moughan, W. C. Smith, G. W. Reynolds and D. J. Garrick. 1993b. The effects of food dry matter intake on endogenous ileal amino acid excretion determined under peptide alimentation in the 50 kg liveweight pig. J. Sci. Food Agric. 62:235-243.

Chung, T. K. and D. H. Baker. 1992. Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: Comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. J. Anim. Sci. 70(12):3781-3790.

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

de Lange, C. F. M., W. C. Sauer, R. Mosenthin and W. B. Souffrant. 1989a. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. 67:746-754.

de Lange, C. F. M., W. C. Sauer and W. B. Souffrant. 1989b. The effect of protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. J. Anim. Sci. 67:755-762.

Dierick, N., I. Vervaeke, J. Decuypere, H. van der Heyde and H. K. Henderickx. 1983. Influence de la nature et du niveau des fibres brutes sur la digestibilite ileale at fecal apparente de la matiere chez les porcs. Rev. Agric. 36:1961-1970.

Donkoh, A., P. J. Moughan and P. C. H. Morel. 1995. Comparison of methods to determine the endogenous amino acid flow at the terminal ileum of the growing rat. J. Sci. Food Agric. 67:359-366.

Donkoh, A. and P. J. Moughan. 1999. Endogenous ileal nitrogen and amino acid flows in the growing pig receiving a protein-free diet and diets containing enzymically hydrolysed casein or graded levels of meat and bone meal. Anim. Sci. 68:511-518.

Han, In. K. and J. H. Lee. 2000. The role of synthetic amino acids in monogastric animal production. Asian-Aus. J. Anim. Sci. 13(4):543-560.

Jagger, B., J. Wiseman, D. J. A. Cole and J. Craigon. 1992. Evaluation of inert markers for the determination of ileal and faecal apparent digestibility values in the pigs. Br. J. Nutr. 68:729-739.

Kavanagh, S., P. B. Lynch, F. O'Mara and P. J. Caffrey. 2001. A comparison of total collection and marker technique for the measurement of apparent digestibility of diets for growing pigs. Anim. Feed Sci. Technol. 89:49-58.

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

Low, A. G. 1982. Digestibility and availability of amino acids from feedstuffs for pigs. A review. Livest. Prod. Sci. 9:511-520.

McCarthy C. H., F. X. Aherne and D. B. Okai. 1974. Use of HCl-insoluble ash as an index for determining apparent digestibility with pigs. Can. J. Anim. Sci. 54:107-109.

Millward, D. J., P. J. Garlick and P. J. Reeds. 1976. The energy cost of growth. Proc. Nutr. Soc. 35:339-349.

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

National Research Council. 1998. Nutrient Requirement of Swine. 10th Ed. National Academy Press. Washington, DC.

Rodwell, V. W. 1985. Catabolism of the carbon skeletons of amino acids. In: Review of Biochemistry, 20th Ed. (Ed. S. Harper). Los Altos. California: Lange Medical Publications. pp. 124-133.

Sauer, W. C, L. Ozimek. 1986. Digestibility of amino acids in swine: Results and their practical application: A review. Livest. Prod. Sci. 15:367-387.

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

Souffrant, W. B. 1991. Endogenous nitrogen losses during digestion in pigs. In: Digestive Physiology in Pigs (Ed. M. W. Verstegen, A. J. Huisman and L. A. den Hartog). Wageningen, Netherlands: EAAP Publication. No. 54:147-165.

SPSS for Windows Base System User's Guide Release 6.0. Marija J. Norusis/SPSS Inc.

Zhang, J. H., D. F. Li., X. S. Piao and Y. C. Zhang. 2000. Determination of ileal apparent amino acids digestibility of feather meal with different inert markers. In: Symposium of 6th Animal Nutrition of China. pp. 622-629.

Zhu, X. P., D. F. Li., S. Y. Qiao, C. T. Xiao, Q. Y. Qiao and C. Ji. 1998. Evaluation of HP 300 soybean protein in starter pig diets. Asian-Australian J. Anim. Sci. 11:201-207.