Asian-Aust. J. Anim. Sci.
Vol. 21, No. 3 : 434 - 442
March 2008

www.ajas.info

The Optimum Methionine to Methionine Plus Cystine Ratio for Growing Pigs Determined Using Plasma Urea Nitrogen and Nitrogen Balance

Shiyan Qiao, Xiangshu Piao, Zhanyu Feng, Yuhua Ding*, Longyao Yue and P. A. Thacker
Ministry of Agriculture Feed Industry Center, China Agricultural University
No. 2 Yuanmingyuan West Road, Beijing 100094, China

ABSTRACT : The objective of this study was to determine the optimum ratio of methionine to methionine plus cystine for growing pigs. A nitrogen balance trial was conducted using a total of 21 barrows (Large White×Landrace) over two replicates. The initial body weight was 20.36±1.22 kg (mean±SD) in the first replicate and 23.54±1.02 kg (mean±SD) in the second. For each replicate, the 21 pigs were randomly assigned to one of seven dietary treatments with three observations per treatment. The diets included a methionine and cystine-deficient basal diet with all other essential nutrients meeting nutrient requirements and six diets formulated with graded levels of DL-methionine (0.00, 0.03, 0.06, 0.10, 0.13, 0.16%) and L-Cystine·HCl·H_2O (0.19, 0.15, 0.11, 0.07, 0.04, 0.00%). This resulted in ratios of methionine to methionine plus cystine of 41.3, 29.6, 35.3, 41.2, 46.0, 51.6 and 57.5%. Each experimental period lasted 12 days consisting of a seven-day adaptation period followed by a five-day total collection of urine and feces. During the collection period, pigs were fed 900 g/day for the first replicate and 1,200 g/day for the second replicate. The feed was provided in three equal portions at 0800, 1500, and 2200 h daily. Pigs had *ad libitum* access to water after feeding. There was a linear ($p<0.01$) and quadratic ($p<0.01$) effect on daily gain and feed conversion as the ratio of methionine to methionine plus cystine increased. Pigs receiving the diets providing a methionine to methionine plus cystine ratio of 51.6% had the best daily gain and feed conversion. Plasma urea nitrogen was also lowest for this treatment. Nitrogen retention increased ($p<0.01$) as the relative proportion of methionine increased up to 51.6% and then a downward trend occurred at 57.5%. The quadratic regression model, as well as one- and two- slope regression line models, were used to determine the optimum ratio of methionine to methionine plus cystine. Eliminating the 35.3% methionine to methionine plus cystine treatment resulted in R^2 values in excess of 0.92. The optimal ratio of methionine to methionine plus cystine was estimated to be 54.15% for nitrogen retention and 56.72% for plasma urea nitrogen. (**Key Words :** Pigs, Methionine, Cystine, Ratio, Nitrogen Retention, Plasma Urea Nitrogen)

INTRODUCTION

The sulfur containing amino acids methionine and cystine are often the third or fourth limiting amino acids in practical diets fed to growing pigs (Russell et al., 1983). Methionine is essential for normal growth as it cannot be synthesized in the body, but cystine can be converted from methionine as needed, hence it is considered dispensable. As a result, the amount of methionine needed in the diet depends on the amount of cystine also present (Chung and Baker, 1992; Yang et al., 1997; Zimmermann et al., 2005). The absolute amounts of methionine and cystine are important but so is the ratio between methionine and cystine. Therefore, nutritionists need to consider not only methionine but also methionine plus cystine requirements when formulating pig diets.

Previous studies with growing pigs have shown that the minimum methionine to methionine plus cystine ratio ranged between 30 and 70% (Wang and Fuller, 1989; Fuller et al., 1989). Part of this variability is due to differences in response criteria (i.e., nitrogen balance vs. growth performance), the bioavailability of the amino acids in the basal diet, and weight of pigs used in the experiments. In a nitrogen balance study with growing gilts (40-80 kg) fed varying ratios of methionine to cystine diets, Reijmers et al. (2002) found the minimum methionine to methionine plus cystine ratio at which protein deposition was maximized was 55%. This value is within the range of values reported in the literature (NRC 1998, Roth and Kirchgessner, 1989).

There is very limited data about the required methionine to methionine plus cystine ratio for maximal protein

* Corresponding Author: Yuhua Ding. Tel: +86-10-6273-3588, Fax: +86-10-6273-3688, E-mail: Dingyh@mafic.ac.cn
Received August 9, 2006; Accepted January 3, 2007

Table 1. Ingredient composition of experimental diets formulated to determine the effects of various methionine to methionine plus cystine ratios on pig performance and nitrogen balance (% as fed)

Ingredients	Methionine to methionine plus cystine ratio						
	41.3	29.6	35.3	41.2	46.0	51.6	57.5
Corn	39.90	39.90	39.90	39.90	39.90	39.90	39.90
Field peas	24.79	24.79	24.79	24.79	24.79	24.79	24.79
Peanut meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Corn starch	8.90	8.71	8.72	8.73	8.73	8.73	8.74
Wheat barn	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Sucrose	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	1.28	1.28	1.28	1.28	1.28	1.28	1.28
Limestone	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Vitamin and mineral premix[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
NaCl	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Choline chloride (50%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Soybean oil	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-lysine HCl (98.5%)	0.49	0.49	0.49	0.49	0.49	0.49	0.49
L-threonine (99%)	0.23	0.23	0.23	0.23	0.23	0.23	0.23
L-valine (99.5%)	0.14	0.14	0.14	0.14	0.14	0.14	0.14
L-isoleucine (99%)	0.14	0.14	0.14	0.14	0.14	0.14	0.14
L-tryptophan (98%)	0.09	0.09	0.09	0.09	0.09	0.09	0.09
DL-methionine (99%)	-	-	0.03	0.06	0.10	0.13	0.16
L-cystine·HCl·H_2O[b] (99.1%)	-	0.19	0.15	0.11	0.07	0.04	-

[a] Provided per kilogram of complete feed: vitamin A, 5,512 IU; vitamin D_3, 2,200 IU; vitamin E, 66.1 IU; riboflavin, 5.5 mg; D-pantothenic acid, 13.8 mg; niacin, 30.3 mg; vitamin B_{12}, 27.6 μg; Mn, 100 mg; Fe, 100 mg; Cu, 234 mg; Zn, 100 mg; I, 0.3 mg; Se, 0.3 mg; Co, 1.0 mg.

[b] 1 kg of L-cystine·HCl·H2O (99.1%) contained 0.851 kg of L-cystine.

deposition in 20 to 30 kg growing pigs. Therefore, the objective of the current study was to establish the optimum dietary ratio of methionine to methionine plus cystine for growing pigs using the nitrogen balance technique and plasma urea nitrogen.

MATERIALS AND METHODS

Animals and diets

A nitrogen balance trial was conducted in the Metabolism Laboratory of the Animal Science and Technology College located on the campus of China Agriculture University (Beijing, China). The trial, conducted in two replicates, utilized 21 barrows (Large White×Landrace) obtained from the Haudu Group (Beijing, China). The initial bodyweight of the pigs averaged 20.36±1.22 kg in the first replicate and 23.54±1.02 kg in the second replicate. In each replicate, the 21 pigs were randomly allocated to one of seven different dietary treatments with three observations per treatment. The basal diet was formulated to meet the requirements for all amino acids except methionine and cystine (NRC, 1998). All other nutrients were formulated to meet or exceed requirements (NRC, 1998). Batches of each feed ingredient were obtained before the start of the study, sampled and analyzed in order to adjust the nutrient composition of the diets.

The content of methionine and cystine in the basal diet was determined to be 0.19 and 0.27%, respectively. Crystalline DL-methionine (0.0, 0.03, 0.06, 0.10, 0.13 and 0.16%) and L-Cystine·HCl·H_2O (0.19, 0.15, 0.11, 0.07, 0.04 and 0.0%) were added to the basal diet by replacing corn starch resulting in seven treatments with ratios of methionine to methionine plus cystine ranging from 29.6 to 57.5% (41.3, 29.6, 35.3, 41.2, 46.0, 51.6 and 57.55%). The ingredient composition of all the diets is presented in Table 1.

For all experimental diets, the vitamin-trace mineral mix and synthetic amino acids were premixed with 10 kg corn before addition to the mixer. A basal mix was manufactured and aliquots of this mix were used to manufacture the final feed.

Experimental procedures

Each replicate consisted of a seven day adjustment period followed by a five day total collection of feces and urine. The pigs were kept in individual metabolic crates and separate collection of feces and urine was accomplished by fitting adhesive feces collection bags onto the back of pigs (Van Kleef et al., 1994). Each stainless steel crate (0.6×0.3 ×0.5 m) was equipped with plastic slotted flooring and contained a 0.25 m^3 round bottom single feeder at the front. The temperature and humidity of the room were controlled within the range of 22 to 25°C and 55 to 70%, using the environmental control system.

The daily ration was divided into three feedings per day, with approximately one third of the ration being fed at

Table 2. Chemical analysis for experimental diets formulated to determine the effects of various methionine to methionine plus cystine ratios on pig performance and nitrogen balance (% as fed)[a]

	Methionine to methionine plus cystine ratio						
	41.3	29.6	35.3	41.2	46.0	51.6	57.5
Chemical analysis							
Dry matter	89.62	90.10	88.98	88.28	89.20	88.94	90.12
Ash	4.83	4.93	5.01	4.81	5.01	4.81	47.56
Crude protein	14.92	15.02	14.82	14.71	15.01	14.68	14.08
Crude fibre	2.28	2.51	2.36	2.29	2.37	2.89	2.40
Ether extract	3.45	3.68	3.81	3.69	3.76	3.72	3.68
Analyzed amino acids							
Arginine	1.18	1.21	1.17	1.11	1.15	1.17	1.18
Cystine	0.27	0.45	0.42	0.37	0.34	0.30	0.27
Histidine	0.34	0.36	0.36	0.35	0.37	0.35	0.36
Isoleucine	0.63	0.62	0.65	0.63	0.63	0.62	0.63
Leucine	1.07	1.11	1.10	1.08	1.09	1.09	1.11
Lysine	1.03	1.10	1.07	1.10	1.02	1.06	1.06
Methionine	0.19	0.19	0.23	0.26	0.29	0.32	0.38
Phenylalanine	0.68	0.67	0.69	0.68	0.66	0.67	0.68
Threonine	0.68	0.68	0.69	0.68	0.70	0.70	0.65
Tryptophan	0.23	0.26	0.20	0.22	0.22	0.23	0.21
Valine	0.82	0.78	0.78	0.76	0.74	0.73	0.79

8,000, 1,500 and 2,200 h. The daily feed allowance of the experimental animals was adjusted according to the feed intake observed in the last three days of the acclimation period. This was the amount of feed that pigs could consume within 20 minutes based on our observations.

From d 4 until the end of the 12-d experimental period, the same amount of feed was fed which exceeded 2.6 times the pig's maintenance energy requirements. This energy intake has been shown not to limit protein deposition (Möhn et al., 2000; De Lange et al., 2001). The feeding rate ranged from 4% to 5.5% of body weight (900 g/d/pig for the first replicate and 1,200 g/d/pig for the second replicate). In the collection period, the wasted feed for each pig was collected, dried and recorded on a dry matter basis.

The animals were weighed at the start of every quantitative feeding period and again at the termination of the trial. Weighing was conduced at 0800 to 0900 h with no feed available. After feeding, water was provided *ad libitum* in the feeding trough.

Sample collection

Feces were collected in the morning, afternoon and evening for five consecutive days taking care to avoid contamination with urine. The total weight of the raw feces for each pig was recorded daily. After collection, feces were placed into labeled plastic bags and frozen at approximately -20°C. At the end of each trial, each pig's daily samples were combined into a single composite sample. From that, a 5% sub-sample was preserved for laboratory analysis. Sub-samples were dried to a constant weight in a forced-air oven at 65°C, equilibrated at room temperature for 24 h, and ground through a 0.45 mm mesh screen.

The urine of individual pigs was collected in plastic containers containing 50 ml of 6 N HCl to maintain the pH of the urine below 3. The total amount of urine excreted by each pig was measured once a day at approximately 1,530 h and recorded on a daily basis. After being filtered through glass wool, a fixed proportion of the urine from each pig was preserved in screw-capped polyethylene containers and frozen at approximately -20°C. When the collection for all five days was completed, each pig's daily samples were thawed and combined into a single sample. A 100 ml composite sample was obtained and then frozen until needed for nitrogen analysis.

At the end of each replicate, 7 ml of blood was collected from the jugular vein of each pig using heparinized vacutainer tubes (Greiner Bio-One Company), approximately 1 h after feeding. All blood samples were chilled and then centrifuged at 3,000×g for 15 min at 4°C within 1 h after collection (Ciji 800 Model Centrifuge, Surgical Instrument Factory, Shanghai, China). An aliquot of plasma was stored at -20°C until analyzed for plasma urea nitrogen.

Chemical analysis

Samples of the feed ingredients were collected before the diets were manufactured, while samples of complete feeds were collected at the start of the trial for analyses. The chemical composition and the amino acid content of all ingredients was analyzed in duplicate in the laboratory of the Ministry of Feed Industry Center (Beijing, China). Moisture, crude protein, crude fiber, ether extract and ash were determined following standard methods (AOAC, 1995).

Table 3. Performance and plasma urea nitrogen for growing pigs fed varying ratios of methionine to methionine plus cystine

	Methionine to methionine plus cystine ratio							SEM[a]	Linear	Quadratic
	41.3	29.6	35.3	41.2	46.0	51.6	57.5			
Weight gain (g/day)	368	375	375	397	395	422	422	36.92	0.01	0.01
Feed intake (g/day)	924	923	923	927	925	924	927	59.36	0.96	0.99
Feed conversion	2.56	2.47	2.51	2.33	2.39	2.20	2.22	0.27	0.01	0.01
Plasma urea nitrogen (mg/dl)	12.50	12.33	12.67	11.83	10.17	9.33	10.17	1.96	0.14	0.33

[a] SEM = Standard error of the mean.

Table 4. Nitrogen balance response for growing pigs fed varying ratios of methionine to methionine plus cystine

	Methionine to methionine plus cystine ratio							SEM[a]	Linear	Quadratic
	41.3	29.6	35.3	41.2	46.0	51.6	57.5			
Nitrogen intake (g/day)	24.79	24.73	26.11	25.60	26.14	26.16	26.07	1.65	0.58	0.80
Fecal nitrogen (g/day)	3.12	3.56	3.54	3.43	3.00	3.19	2.81	0.48	0.18	0.41
Urinary nitrogen (g/day)	9.62	8.85	10.49	7.88	7.66	6.91	7.88	1.29	0.17	0.37
Retained nitrogen (g/day)	12.05	12.32	12.09	14.29	15.49	16.05	15.38	1.23	0.01	0.01
Nitrogen retained (%)	48.61	51.07	46.64	56.61	60.00	62.05	59.57	4.53	0.02	0.05
Nitrogen digestibility (%)	88.74	86.01	86.62	86.76	88.48	88.09	89.30	1.38	0.04	0.13

[a] SEM = Standard error of the mean.

The amino acid content of the diets was determined by High Performance Liquid Chromatography (Hitachi L-8800 Amino Acid Analyzer, Tokyo, Japan). All samples were hydrolyzed for 24 h at 110°C with 6 N HCl prior to analysis. Sulfur-containing amino acids were analyzed after cold formic acid oxidation for 16 h before acid hydrolysis. Tryptophan was determined after alkaline hydrolysis (4 N NaOH) for 22 h at 110°C. The chemical composition of the diets is listed in Table 2.

Plasma urea nitrogen was determined on a fully automatic Biochemical Analyzer (Technicon RA 1000) and by enzymatic UV test (Ureaza method/GLDH) based on the report of Kerschner and Ziegenhorn (1985). A urea kit produced by Zhong Sheng Beikong Bio-technology and Science Inc. (Beijing, China) was used for this analysis. Fecal and urinary nitrogen were analyzed with a semi-automatic analyzer (Kjeltec^{TM} 2100 Distillation Unit) by the Kjeldahl method (AOAC, 1990).

Statistical analysis

Data from the two replicates were analyzed using the General Linear Model (GLM) procedure of the SAS statistical package (SAS, 2002) using the pig as the experimental unit. The experimental data were subjected to analysis of variance using a model that included the effect of diet and the two replicates. The results were considered significant if $p<0.05$.

The optimal ratio between methionine and methionine plus cystine of the growing pigs was estimated with a quadratic regression model as well as one- and two- slope regression line models (Coma et al., 1995a) using nitrogen retention and plasma urea nitrogen as the dependent variables regressed against dietary level of methionine to methionine plus cystine ratio. The appropriate GLM and NLIN procedures of SAS (2002) were used for these estimates. The applied quadratic model was:

$$Y = b_0+b_1X+b_{11}X^2$$

Where Y = the response parameter (nitrogen retention, plasma urea nitrogen) and X_1 = the ratio of methionine to methionine plus cystine b_0, b_1, b_{11} = the coefficients of the equation. The ratio at which the response reached 95% of the maximum response was estimated as the optimal value.

The regression of the one-slope and two-slope models used in the present experiment are described as follows:

$$Y = L+U\ (R-X_{LR});$$

$$Y = L+U\ (R-X_{LR})+V(X_{GR}-R)$$

Where L = the ordinate; R = the abscissa of the breakpoint (the estimated requirement). X_{LR} means X less than R; X_{GR} means X greater than R. U = the slope of the line at X<R, and V = the slope of the line at X>R. By definition, $(R-X_{LR})$ is zero when X greater than R, and $(X_{GR}-R)$ is zero when X less than R. The ratio at which the breakpoint was achieved was estimated as the optimal value (Robbins et al., 1979; Coma et al., 1995a). The mean square error (MSE) and the coefficient of determination (R^2) were used to assess the goodness of fit for the different models (Coma et al., 1995a).

RESULTS

The results showed no significant replicate×treatment interaction ($p>0.05$) for any of the studied variables. Therefore, data from the two replicates were pooled for analysis.

Since the level of feed intake was controlled, feed intake

Table 5. Asymptotic characteristics of plasma urea nitrogen and nitrogen retention responses to relative proportions of methionine to methionine plus cystine

Variable	Model	Requirement	R^2	MSE
Nitrogen retention	Quadratic	54.15	0.99	0.095
	One-slope broken line	51.24	0.96	0.192
	Two-slope broken line	53.96	0.98	0.159
Plasma urea nitrogen	Quadratic	56.17	0.92	0.563
	One-slope broken line	53.94	0.94	0.233
	Two-slope broken line	56.72	0.99	0.113

was similar among all dietary treatments. As the relative proportion of methionine to methionine plus cystine increased from 29.6 to 57.5%, average daily gain and feed conversion improved linearly ($p = 0.01$) and quadratically ($p = 0.01$). The poorest weight gain and feed utilization was observed for pigs fed the basal diet. The best daily gain and feed conversion was observed for pigs fed the diet in which the methionine to methionine plus cystine ratio was 51.6%. For plasma urea nitrogen, the lowest and highest values occurred for pigs fed the 51.6 and 35.3% methionine to methionine plus cystine ratio diets (Table 3).

Increasing the relative proportion of methionine to methionine plus cystine resulted in a significant linear ($p = 0.02$) and quadratic ($p = 0.05$) increase in nitrogen retention (Table 4). Nitrogen digestibility increased linearly ($p = 0.04$) with increased proportions of methionine to methionine plus cystine.

Three statistical models were fitted to the nitrogen retention and plasma urea nitrogen data (Tables 3 and 4). Based on the nitrogen retention response to the ratio of methionine to methionine plus cystine, three regression equations were obtained using the quadratic regression, one- and two- slope regression models, respectively:

$$Y = -18.19+1.20X-0.011X^2$$

$$Y = 15.72-0.26\times(51.24-X_{LR})$$

$$Y = 16.42-0.26\times(53.96-X_{LR})-0.10\times(X_{GR} - 53.96)$$

Based on the corresponding equations, the optimal ratios of methionine to methionine plus cystine were determined to be 54.15, 51.24 and 53.96%, respectively.

When plasma urea nitrogen was considered as the dependent variable, the optimal ratios of methionine to methionine plus cystine were estimated to be 56.17, 53.94 and 56.72%. The corresponding regression equations were listed as followed:

$$Y = 33.951-0.816\ X-0.0069\ X^2$$

$$Y = 9.75+0.19\times(53.94-X_{LR})$$

$$Y = 9.22+0.19\times(56.72-X_{LR})-0.13\times(X_{GR}-56.72)$$

DISCUSSION

In the present study, the optimal ratio of methionine to methionine plus cystine was estimated in 20 to 30 kg growing pigs using the nitrogen balance technique. Based on our design, the six test diets contained varying levels of methionine and cystine but the total content of methionine plus cystine was similar across treatments and was close to the value recommended by the NRC (1998). Moreover, all other essential nutrients, especially energy and other amino acids were designed to be at or above requirement (NRC, 1998).

For growing animals, amino acids are basically used for protein accretion and maintenance and deposited protein relies on the level of the first limiting amino acid. Assuming our dietary formulation was accurate and the methionine plus cystine content of the test diets did not exceed the requirements of growing pigs, whole-body protein synthesis should theoretically occur at a level determined by the optimal ratio of methionine to cystine. If the dietary ratio of methionine to cystine is below the optimal value, more cystine and less methionine will be consumed. Protein synthesis will be determined by the level of dietary methionine, which leads to less protein deposition. When the ratio in the test diets is above the value, methionine will be in relative excess and cystine will be in relative deficiency. However, the deficiency in cystine can be overcome by conversion from methionine via the trans-sulfuration pathway. In fact, cysteine (1/2 cystine) is the genuine element used to incorporate into protein. Cystine (the dimmer form of cysteine), is produced when cysteine is in solution (Lewis, 2003). Because of the molecular weight difference between methionine and cysteine, the efficiency of methionine in meeting the biological need for cysteine on a weight basis is 80% (Chung and Baker, 1992). Thus, an excess of methionine is not sufficient to make up for a deficiency in cystine in this condition. Protein synthesis will also be reduced due to the low cystine intake. From this, it can be concluded that increasing the relative proportion of methionine will result in greater protein synthesis until the optimal ratio of methionine to cystine is attained, and subsequently, when the relative proportion of methionine is above its optimal value, protein synthesis will be reduced with further increases in methionine intake.

Because of conversion from methionine, a deficiency of cystine will lead to relatively less of a change in protein synthesis than a deficiency of methionine in the presence of a constant methionine plus cystine content. This has been confirmed by Roth and Kirchgessner (1989). In a similar experiment for 30 to 60 and 60 to 90 kg pigs, they found that pigs with a predominant proportion of methionine obtained higher performance than those with a predominant proportion of cystine. Here, the parameters of performance reflect the status of protein synthesis. According to the current experimental design and statistical analysis, when the optimal ratio of methionine to cystine is fed, maximal protein synthesis occurs.

The status of protein synthesis can be measured using biological response criteria such as growth, nitrogen retention and plasma urea. In fact, an inherent relationship exists between several criteria. Nitrogen retention is a direct indicator of protein synthesis. Here, protein deposition (synthesis) can be calculated as nitrogen retained×100/16 (Möhn et al., 2000). For young pigs with minimal fat deposition, growth is almost directly proportional to lean tissue deposition which primarily relies on protein deposition.

When protein synthesis is limited due to an unsuitable ratio of methionine to cystine, excess amino acids (including methionine or cystine) are catabolized to their metabolic end-products which for all amino acids include bicarbonate and ammonia. Ammonia enters the nitrogen pool of the body and is excreted primarily as urea in mammals. The status of urea in the body is therefore reflected by plasma urea. The measurement of these excreta provides an indirect and inverse measurement of changes in protein synthesis. As the relative proportion of methionine increases towards its optimal value, more protein is synthesized which leads to increased nitrogen retention, improved animal performance and decreased plasma urea. The inverse changes of nitrogen retention, animal performance and plasma urea occur when there is a continuous increase in the relative proportion of methionine from its optimal value. The inverse relationship between plasma urea (nitrogen) and lean growth (growth for young pigs) was also detected in previous reports published by Coma et al. (1995b).

So, based on maximal nitrogen retention or minimal plasma urea, the optimal ratio of methionine to cystine can be determined for the growing pigs in the present study. This estimation can be conducted by applying suitable statistical modeling techniques to the chosen biological response. We observed nitrogen retention and plasma urea nitrogen exhibited an anticipative change tendency from 35.3 to 57.5% methionine to methionine plus cystine (Tables 3 and 4). However, nitrogen retention and plasma urea nitrogen in the 29.6% treatment were superior to those in the 35.3% methionine to methionine plus cystine treatments, which meant that an increase in the relative proportion of methionine towards its optimal value caused a decrease in those variables. The reason for this is not known.

The quadratic and broken-line regression analyses were used to determine the relation between methionine and cystine in our study. For nitrogen retention and plasma urea nitrogen, when the values generated from the treatment for 29.6% of methionine to methionine plus cystine were removed, those models fitted the data very well.

Using the quadratic regression, as well as the one- and two- slope broken-line regression models, the required ratios of methionine to methionine plus cystine were estimated to be 54.15, 51.24 and 53.96% for nitrogen retention. However, when considering the plasma urea nitrogen variable, using the corresponding statistical models, the required ratios of methionine to methionine plus cystine were estimated to be 56.17, 53.94 and 56.72%. Obviously, the plasma urea nitrogen assay resulted in higher values than the nitrogen retention assay using the corresponding statistical models.

These differences may be attributed to an imbalance of electrolytes in the diets, where the chloride existing in the crystalline cystine (L-Cystine·HCl·H_2O) would tend to decrease the cation:anion ratio. Several reports in pigs have indicated that a diet with excess anion or chloride resulted in markedly lower plasma urea nitrogen concentrations (Slagle and Zimmerman, 1979; Honeyfield et al., 1985). However, total nitrogen excretion in pigs was found to be constant, although the excess cation intake resulted in significantly greater urea excretion (Cai et al. 1992). This was explained by Welbourne et al. (1986) who suggested that with the maintenance of acid-base balance in the body, urea was isochronously synthesized with ammonia production so that nitrogen excretion remained constant. Thus, the addition of various levels of L-Cystine-HCl in the test diets influenced plasma urea nitrogen but not nitrogen retention, which may have produced the difference between the estimated results from the two variables. So the result from the nitrogen retention assay is more reasonable and acceptable.

For the nitrogen retention response, we found that the quadratic regression model had lower MSE and higher R^2 than either of the broken-line regression models (Figures 1-3). So the nitrogen retention response is better described by the quadratic regression model than by the broken-line regression models. Therefore, the determined value 54.15% is considered to be the estimated required ratio methionine to methionine plus cystine according to the nitrogen retention response. Similarly, for the plasma urea nitrogen response, we observed that the two-slope broken-line regression model tended to fit the data better than the other two regression models. The determined value 56.72% is

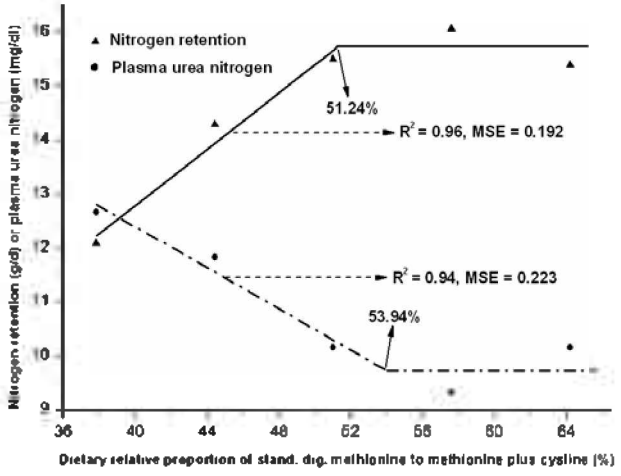

Figure 1. Use of the one-slope, broken-line regression model to describe the responses of nitrogen retention and plasma urea nitrogen to the proportion of methionine to methionine plus cystine in 20-50 kg growing pigs.

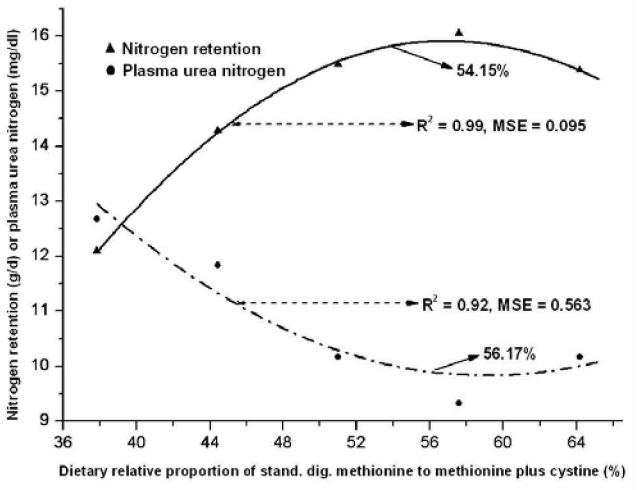

Figure 3. Use of the quadratic model to describe the responses of nitrogen retention and plasma urea nitrogen to the relative proportion of methionine to methionine plus cystine in 20-50 kg growing pigs.

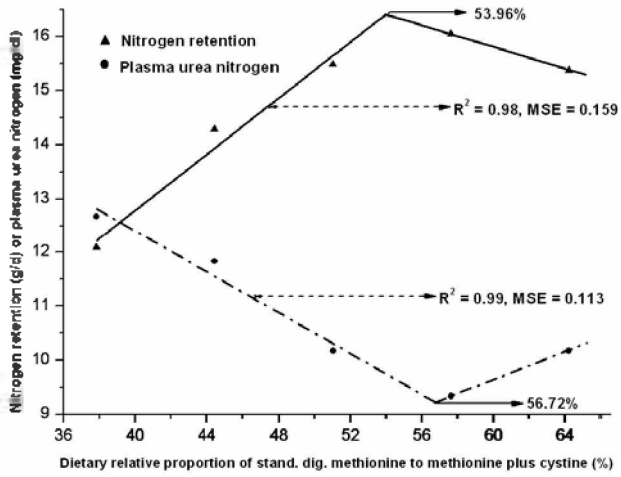

Figure 2. Use of the two-slope, broken-line regression model to describe the responses of nitrogen retention and plasma urea nitrogen to the relative proportion of methionine to methionine plus cystine in 20-50 kg growing pigs.

considered to be the estimated required ratio when the plasma urea nitrogen response was considered (Figures 1-3).

Referring to the dietary amino acid requirements for 20 to 50 kg growing pigs (NRC 1998), the recommended methionine and methionine plus cystine levels are 0.25% and 0.54%, respectively. Therefore, the ratio of methionine to methionine plus cystine is 46.29%. Obviously, our determined value is higher than this value. However, in a recent nitrogen balance experiment for 40 to 80 kg growing gilts, Reijmers et al. (2002) found the ratio of digestible methionine to methionine plus cystine for maximal body protein deposition was 55%. This value is very close to our evaluation of 54.15% based on maximal nitrogen retention.

These results agree with the studies conducted by Schutte et al. (1991) and Chung and Baker (1992), who indicated in growing pigs (respectively 20 to 50 kg and 10 to 20 kg) that methionine should contribute more than 50% of the total methionine and cystine requirement. In a previous growth assay for 30 to 60 kg and 60 to 90 kg pigs, Roth and Kirchgessner (1989) found that the ratio of methionine to methionine plus cystine, at maximal weight gain or feed efficiency, was more than 55%. This is somewhat higher than either of our estimated values. Several factors may have contributed to the differences in the relative proportion of methionine to methionine plus cystine estimates of growing pigs in the above studies including: 1) use of a different experimental design, i.e. nitrogen balance vs. growth assay, 2) use of different response criteria, i.e., nitrogen retention, plasma urea nitrogen or performance, 3) differing methionine plus cystine content employed in the diets, In addition, young animals use more amino acids for protein accretion than for maintenance compared with older ones. Protein accretion in pigs requires a greater proportion of methionine (Fuller et al., 1989; Mahan and Shields, 1998), while maintenance in pigs requires a greater proportion of cystine (Fuller et al., 1989; NRC, 1998). So the estimated result may also be influenced by age of pig.

For the present experiment, there are some additional factors which may have affected our results. In our design, the test diets were provided with constant levels of methionine plus cystine and varying levels of methionine and cystine. Lewis (2003) indicated that the molecular weight of methionine (149) is greater than that of cysteine (121), and equal weights of these two amino acids provide only 81% as many moles of methionine as cysteine (121/149 = 0.81). Thus, on a weight basis, increasing the

methionine to cystine ratio provides a decreasing number of moles of sulfur containing amino acids. In our study, the content of methionine plus cystine was not constant when expressed on a molar basis, which possibly influenced the results to some extent. However, we found that previous experiments also ignored this effect (Roth and Kirchgessner, 1989; Reijmers et al., 2002).

In practical swine diets containing sufficient amounts of the sulfur amino acids, generally cystine is more in excess than methionine. A high cystine intake increased the requirement of methionine plus cystine in pigs, but there was no evidence that excess cystine interferes with methionine (Lewis, 2003). When low protein diets are used in young pigs, perhaps methionine will be lacking. In this case, methionine should be added to meet its requirement, even though methionine plus cystine may appear to be adequate.

CONCLUSION

In the present nitrogen balance trial with an equal feed intake, nitrogen retention and plasma urea nitrogen variables were used to determine the optimum methionine to methionine plus cystine ratio. The data from the two variables were analyzed to fit a quadratic regression, as well as one- and two- slope regression models. By comparing the estimated results from three regression models, the two most precise values, 54.15 and 56.72%, were concluded to be the optimal relative proportion of methionine for nitrogen retention and plasma urea nitrogen responses, respectively. Due to the influence of added crystalline cystine on plasma urea nitrogen, the value 54.15% estimated by nitrogen retention assay, was considered to be the more reasonable result.

REFERENCES

AOAC. 1995. Official Methods of Analysis, 16th ed. Association of Official Analysis Chemists, Washington, DC.

Cai, Y. J., R. C. Ewan and D. R. Zimmerman. 1992. Effects of dietary energy, protein and potassium levels on plasma urea nitrogen and free amino acids in finishing pigs. J. Anim. Sci. 70(Suppl. 1):236(Abstr.).

Chung, T. K. and D. H. Baker. 1992. Maximal portion of the young pig's sulfur amino acid requirement that can be furnished by cystine. J. Anim. Sci. 70:1182-1187.

Coma, J., D. Carrion and D. R. Zimmerman. 1995a. Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs. J. Anim. Sci. 73:472-481.

Coma, J., D. R. Zimmerman and D. Carrion. 1995b. Relationship of rate of lean tissue growth and other factors to concentration of urea in plasma of pigs. J. Anim. Sci. 73: 3649-3656.

De Lange, C. F. M., A. M. Gillis and G. J. Simpson. 2001. Influence of threonine intake on whole-body protein deposition and threonine utilization in growing pigs fed purified diets. J. Anim. Sci. 79:3087-3095.

Fuller, M. F., R. McWilliam, T. C. Wang and L. R. Giles. 1989. The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. Br. J. Nutr. 64:255-267.

Honeyfield, D. C., J. A. Froseth and R. J. Barke. 1985. Dietary sodium and chloride levels for growing-finishing pigs. J. Anim. Sci. 60:691-698.

Kerschner, L. and J. Ziegenhorn. 1985. Urea. In: Methods in Enzymatic Analysis. 3rd edn. (Ed. H. U. Bergmeyer). Vol. 8, Verlag Chemie, Weinheim, pp. 444-453.

Lewis, A. J. 2003. Methionine-cystine relationships in pig nutrition. In: Amino Acids in Animal Nutrition., 2nd edn. (Ed. J. P. F. D'Mello) vol. 8, CAB International, Wallingford, UK pp. 143-155.

Mahan, D. C. and R. G. Jr. Shields. 1998. Essential and nonessential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. J. Anim. Sci. 76:513-521.

Möhn, S., A. M. Gillis, P. J. Moughan and C. F. M. de Lange. 2000. Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. J. Anim. Sci. 78:1510-1519.

NRC. 1998. Nutrient Requirements of Swine, 10th ed. National Academic Press, Washington, DC.

Reijmers, A. T. H., A. M. Gillis and C. F. M. de Lang. 2002. Optimum dietary methionine to methionine+cystine ratio for growing pigs. In: Amino Acids in Animal Nutrition (Ed. M. Pack, J. Ficklen, M. Rademachen, A. Lemme, S. Mack, D. Hohlen, J. Fonlaine and A. Petoi), CORAL SANIVET, Bucharest pp. 453-457.

Robbins, K. B., H. W. Norton and D. H. Baker. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109:1710-1714.

Roth, F. X. and M. Kirchgesser. 1989. Influence of the methionine:cysteine relationship in the feed on the performance of growing pigs. J. Anim. Physiol. Anim. Nutr. 61:265-274.

Russell, L. E., G. L. Cromwell and T. S. Stahly. 1983. Tryptophan, threonine, isoleucine and methionine supplementation of a 12% protein, lysine-supplemented, corn-soybean meal diet for growing pigs. J. Anim. Sci. 56:1115-1123.

SAS. 2002. SAS/STAT at User's Guide (Release 9.2.0.1.0 Ed.). SAS Inst. Inc., Cary, NC.

Schutte, J. B., M. W. Bosch, J. de Jong, E. J. van Weerden and F. Koch. 1991. Factors affecting the requirement of dietary sulphur-containing amino acids of young pigs. Netherland J. Agric. Sci. 39:91-101.

Slagle, S. P. and D. R. Zimmerman. 1979. Evaluation of a yeast single cell protein with young pigs. J. Anim. Sci. 49:1252-1260.

Van Kleef, D. J., K. Deuring and P. van Leeuwen. 1994. A new method of faeces collection in the pig. Lab. Anim. 28:78-79.

Wang, T. C. and M. F. Fuller. 1989. The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. Br. J. Nutr. 62:77-89.

Welbourne, T. C., V. Phromphetcharat, G. Givens and S. Joshi. 1986. Regulation of interorganal glutamine flow in metabolic acidosis. Am. J. Physiol. 250(4 Pt 1):E457-E463.

Yang, C. J., D. W. Lee, I. B. Chung, Y. M. Cho, I. S. Shin, B. J. Chae, J. H. Kim and In K. Han. 1997. Developing model equation to subdivide methionine+cystine requirements into requirement for growth and maintenance in pigs. Asian-Aust. J. Anim. Sci. 10(1):86-97.

Zimmermann, B., R. Mosenthin, M. Rademacher, P. B. Lynch and E. Esteve-Garcia. 2005. Comparative studies on the relative efficacy of DL-methionine and liquid mechionine hydroxy analogue in growing pigs. Asian-Aust. J. Anim. Sci. 18(7):1003-1010.