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Effect of source of methionine in broken rice-soybean diet on production performance, blood chemistry, and fermentation characteristics in weaned pigs

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ABSTRACT: This study was conducted to compare the effect of source of methionine (Met) in broken rice-soybean diet on performance, blood biochemistry, and fermentation characteristics in weaned pigs. Forty-eight male crossbreed pigs (BW 11 ± 0.1 kg) were randomly allocated to three groups with four replications in a completely randomized design. The experimental diets were: (1) basal diet without methionine (Control; total sulfur amino acids (TSAA) 0.60%); (2) basal diet supplemented with DL-methionine (DLM) (TSAA 0.76%); and (3) basal diet supplemented with DL-2-hydroxy-4-(methylthio) butanoic acid (LMA) (TSAA 0.76%). Supplementation with DLM and LMA improved growth performance of piglets and decreased blood urea nitrogen and increased serum albumin ($P < 0.01$). The population of *Lactobacillus* spp. in the caecum was decreased by both DLM and LMA supplementation ($P < 0.05$). Succinic acid concentration in the caecum of pigs fed the DLM diet was greater than that of LMA group ($P < 0.05$). It can be concluded that LMA can be used as a good source of Met (88% bioefficacy, weight/weight) in broken rice-soybean diet, although the serum albumin and fermentation characteristics (succinic acid) in the gastrointestinal tract were different.

Keywords: methionine; short-chain fatty acids; piglets

INTRODUCTION

In Southeast Asia, broken rice (by-product from rice processing) is the major feed ingredient used in weanling pig diets because rice production in this region is about 32.32% of world rice production. Nutritional contents in broken rice are comparable with those in corn, but the risk of mycotoxins contamination is lower. Moreover, Alcantara et al. (1989) reported that pigs that were fed 20% rough rice in place of corn had increased growth rate, feed efficiency, and apparent digestibility of protein and energy.

Methionine (Met) is often the second or third limiting amino acid in pig diet. Synthetic Met is often added to diets for young pigs. In corn-soybean based diets, synthetic Met is not commonly used in the growing and finishing periods but this synthetic amino acid may be added to diets for young pigs (Gaines et al. 2005; Bauchart-Thevret et al. 2009). In terms of amino acids supplementation, however, the feeding value of broken rice is rela-

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tive to corn. The effect of synthetic Met has not been reported.

DL-Methionine (DLM) is the feed industry standard for Met addition. Recently, liquid methionine hydroxy analog free acid (LMA) has increasingly been used. Reports on the bioefficacy of the two forms have been contradictory. For example, Kim et al. (2006) reported that the bioefficacy of the LMA to DLM was 63% on a weight/weight basis, or 73% on an equimolar basis in pigs. However, several other investigators reported 88% bioefficacy on an equimolar basis of LMA compared to DLM in pigs (Chung and Baker 1992; Knight et al. 1998; Yi et al. 2006).

Liquid methionine hydroxy analogue is a short-chain (C4) monocarboxylic acid with a hydroxy group on the alpha-carbon and pK_a between 3 and 4, so it is an organic acid until converted to Met within the body (Dibner and Buttin 2002). Due to the property of organic acids, Kaewtapee et al. (2010) reported that adding LMA in drinking water reduced the total plate count and *Escherichia coli* (*E. coli*) in the water and consequently improved growth performance of piglets. In poultry, Poosuwan et al. (2007) demonstrated that the minimum inhibitory concentration (MIC) of LMA for *E. coli* was 0.24% v/v in water, and this level promoted growth performance and tended to reduce *E. coli* in the gastrointestinal tract of broiler chicks. However, the published information on the effect of these two Met sources on gastrointestinal pH, microbial population, and short-chain fatty acids (SCFA) (end products of microbial activities) of young pigs is limited.

Total serum protein, globulin, and albumin have been used to indicate both protein quantity and quality (Eggum et al. 1989). In humans, normally increases high protein quality serum albumin, since highly available amino acids increased albumin synthesis (Thalaccker-Mercer et al. 2007). Accordingly, Poosuwan et al. (2010) reported that high protein diet (18% crude protein (CP)) in laying hens significantly increased egg production, serum albumin, and alpha-globulin compared with the low protein diet (14% CP). Although Met supplementation commonly increases the balance of amino acids in diet, comparative effects of DLM and LMA on serum proteins have not been reported.

Based on 88% bioefficacy of LMA if compared to DLM, it was hypothesized that growth performance of piglets fed diets containing LMA may be equal to that of pigs fed diets supplemented with DLM, but serum protein and fermentation characteristics in the gastrointestinal tract may be different. Therefore, this study was conducted to evaluate effects of dietary Met sources (DLM and LMA) in piglets fed broken rice-soybean diets on growth performance, blood characteristics, and fermentation characteristics in the gastrointestinal tract.

MATERIAL AND METHODS

Experimental animals and management. This study was conducted at the Animal Research Farm, Department of Animal Science, Kasetsart University, Thailand. Pigs were kept, maintained, and treated in accordance with the Guide for the Care and Use of Laboratory Animals, Secretariat Office of the National Committee for the Research of Animal Development, National Research Council of Thailand. Forty-eight male crossbred pigs (Large White × Landrace × Duroc, 11.1 ± 0.1 kg body weight, uniformity = 99.01% and CV = 0.90%) at six weeks of age were housed in group pens (1.0 m^2/pen, 4 pigs/pen, 4 replicate pens/treatment) for 6 weeks with evaporative cooling systems to maintain environmental temperature at an average of 27.98 ± 1.80°C. Each pen was equipped with two nipple drinkers and two automatic feeders. Pigs were offered feed and water *ad libitum*. Initial body weight and final body weight were measured to calculated body weight gain, average daily gain (ADG), and feed conversion ratio (FCR). Feed intake was recorded daily on a per pen basis.

Dietary treatments. Two Met sources, powder form (DL-methionine 99%; DLM) and liquid form (DL-2-hydroxy-4-(methylthio) butanoic acid 88%; LMA), were provided by Sumitomo Chemical Co., Ltd. (Tokyo, Japan). There were three treatments; each treatment consisted of 4 replications per 4 piglets. A complete randomized design was used.

All nutrients in the basal diet (except Met) met the requirements according to National Research Council (1998). The basal diet was formulated to contain 20% CP, 1.35% total lysine (Lys), 0.37% total Met, 0.65% total Met+cysteine (Cys), and 14.03 MJ ME/kg. The main energy source in the diets was broken rice. Chemical analysis of nutrients in the basal diet (Table 1) shows that Met and total sulfur amino acids (TSAA) levels were 0.27 and 0.60%

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Table 1. Feed ingredients and chemical composition of the basal diet (as-fed basis)

Ingredient	Amount (%)
Broken rice	43.08
Corn	5.00
Full rice bran (stabilized)	8.15
Soybean meal (48% CP)	4.00
Full fat soybean (extruded)	27.41
Fish meal (60% crude protein)	2.00
Soybean oil	1.29
L-Lysine HCL	0.35
L-Threonine	0.10
Dried skim milk (33% crude protein)	5.00
Monodicalcium phosphate (21% P)	1.83
Calcium carbonate	1.09
Salt	0.20
Premix[1]	0.50
Total	100.00
Calculated chemical composition[2]	
Metabolizable energy (MJ/kg)	14.03
Crude protein (%)	20.00 (19.87)
Ether extract (%)	8.00 (6.98)
Ash (%)	6.90 (5.92)
Calcium (%)	1.00 (0.97)
Available phosphorus (%)	0.50
Lysine (%)	1.35 (1.29)
Methionine + cysteine (%)	0.65 (0.60)
Methionine (%)	0.37 (0.27)
Threonine (%)	0.85 (0.72)

[1]contents per kg of diet: vitamins: A 2 000 000 IU, D_3 320 000 IU, E 12 000 IU, K_3 700 mg, B_1 300 mg, B_2 1600 mg, B_6 375 mg, B_{12} 7 mg, nicotinic acid 10 000 mg, pantothenic acid 5000 mg, folic acid 220 mg, biotin 20 mg, choline 30 000 mg; minerals: Fe 22 500 mg, Cu 20 000 mg, Mn 7500 mg, Zn 20 000 mg, Co 100 mg, I 200 mg, Se 300 mg

[2]analyzed values in brackets

of diet, respectively. Therefore, Met and TSAA concentrations in the basal diet were by 10 and 8% lower, respectively, than recommendations of NRC (1998) (Table 2). The TSAA : Lys ratio in the supplemented diets was 60% which is in accordance with the recommendation of Gaines et al. (2005).

The dietary treatments consisted of three groups: (1) basal diet (Met = 0.27%, TSAA = 0.60%); (2) basal diet + 0.16% DLM; (3) basal diet + 0.18% LMA (Table 2). The concentrations of Met and TSAA in the basal diet were based on analyzed values. Calculation of the amount of LMA for supplementation of the basal diet was based on 88% bioefficacy of DLM (weight/weight) (Yi et al. 2006). The pH of each of the experimental diets was measured by mixing a 10 g sample with 90 ml distilled water (pH 7) for 10 min at room temperature (Roth and Kirchgessner 1998) and use of a pH meter (IQ 150 with micro probe PH-17SS; IQ Scientific Instruments, Inc., Carlsbad, USA). The volume of supplemented Met sources in the basal diet is shown in Table 2.

Determination of gastrointestinal pH. At the end of the trial, four pigs per treatment were euthanized using CO_2 asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO_2). Immediately thereafter the pH of the contents of the gastrointestinal tract including stomach, duodenum, jejunum, ileum, caecum, colon, and rectum were directly measured using a pH meter.

Bacterial counts in the hindgut. Microbiological enumeration was performed in samples from the hindgut (caecum and rectum). The contents were collected immediately after asphyxiation into sterile centrifuge tubes, placed on ice, and transported (within 1 h after collection) to the laboratory for selected bacterial enumeration. The bacterial count in the samples was conducted according to the procedures described by Brown (2005). Briefly, approximately 5 g of sample was diluted with 45 ml of 1% peptone solution (Oxoid

Table 2. Methionine sources and corn starch supplements to the basal diet

	Control (Met-deficient diet)[1]	DLM[2]	LMA[3]
Supplementary Met (mg/100 g)	0	0.16	0.18
Corn starch (mg/100 g)	0.18	0.02	0

Met = methionine, TSAA = total sulfur amino acids, DLM = DL-methionine, LMA = liquid methionine hydroxy analog free acid
Met and TSAA levels in basal diet were based on analyzed values
[1]Met = 0.27%, TSAA = 0.60%; [2]Met = 0.43%, TSAA = 0.76%; [3]Met = 0.43%; TSAA = 0.76%

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Laboratories, Basingstoke, UK). A tenfold serial dilution was used to reduce the concentration to 1 : 10, and 0.1 ml was applied onto duplicate agar plates for each dilution. *Lactobacillus* spp. and *E. coli* grew on De Man, Rogosa, and Sharpe (MRS) agar (Difco™; Becton, Dickinson and Co., Sparks, USA) and MacConkey agar (Oxoid Laboratories), respectively. Using the spread plate technique to determine the number of bacteria, all agar plates were incubated for 24 h at 37.0°C, and after removal from the incubator, *Lactobacillus* spp. and *E. coli* colonies were immediately determined per plate by counting.

Analysis of short-chain fatty acids. A 1.5 ml aliquot of samples from each caecum were centrifuged (model MX-301; TOMY Kogyo, Tokyo, Japan) in microcentrifuge tubes at 14 000 *g*, 4°C for 10 min. The samples were acidified to pH 3 using concentrated H_2SO_4. Briefly, a 360 µl aliquot of each sample was transferred to a microcentrifuge tube containing 40 µl of acid solution (50mM H_2SO_4 and 100mM quinic acid as an internal standard). After mixing by vortex for 30 s and standing at room temperature for 10 min, the centrifugation was repeated (14 000 *g*, 4°C for 10 min) and the supernatants were analyzed for SCFA with the high performance liquid chromatography (HPLC).

The HPLC system consisted of a Waters Alliance model e2695 Separations Module (Waters Corporation, Milford, USA), Aminex HPX-87H Ion Exclusion Column (7.8 mm i.d. × 330 mm) (Bio-Rad, Richmond, USA), a Micro-Guard Cation-H guard column (4.6 mm i.d. × 30 mm) (Bio-Rad), and 2998 Photodiode Array Detector (Waters Corporation). The supernatants were filtered with a 0.22 µm nylon syringe filter 13 mm (No. 2166) (Alltech Associates Inc., Deerfield, USA) according to the method of Fernandes et al. (2000), 20 µl of each sample was injected into the HPLC with autosampler and 0.005M H_2SO_4 as the mobile phase. The running conditions provided for column heat of 60°C, flow rate of 0.6 ml/min, and absorbance detector operating at a wavelength of 210 nm. A mixture of formic, acetic, propionic, butyric, valeric, succinic, and lactic acids was included as a standard in all analyses. The acid peaks were detected by the Empower 2™ software (Build 2154, 2005) at a wavelength of 210 nm. Qualitative acid analysis was determined by the retention time of the acid peaks, while quantitative analysis was carried out using a standard curve composed of the various acid concentrations vs the peak area ratio of the acid peaks and the internal standard.

Blood collection and analyses. On the final day of experiment, five pigs per treatment with medium body weight were selected from each group and a 3 ml blood sample was collected to plastic tubes without anticoagulants via the jugular vein (feeders were removed from pens 3 h before). Samples were centrifuged at 2500 *g* for 10 min at room temperature within 1 h after collection. Serum was harvested and stored at –20°C until analyses for total protein, albumin, globulin, and blood urea nitrogen by commercial test kits (Assay kit; HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). Total protein concentration in the serum was analyzed by Biuret method (photometric colourimetric test), serum albumin concentration was analyzed by BCG-method (photometric colourimetric test), and serum blood urea nitrogen concentration was analyzed by urea liquid colour method (enzymatic colourimetric test). The globulin values were calculated by subtracting the values of albumin from the corresponding values of total protein (Meyer and Harvey 2004).

Statistical analysis. Data were analyzed as a completely randomized design using the ANOVA procedures of SAS (Statistical Analysis System, Version 9.0, 2002). The model used was as follows:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where

Y_{ij} = dependent variable

μ = overall mean effect

τ_i = fixed effect of treatments

I = control, DLM, LMA

ε_{ij} = residual experimental error with $N(0, \sigma^2)$

The significance of the differences between the treatment group means for each parameter was evaluated using the Duncan's New Multiple Range Test (DMRT). Probabilities of $P < 0.05$ and $P < 0.01$ were taken to indicate significant differences. All statistical analyses were computed in accordance with the method of Steel and Torrie (1980).

RESULTS

Growth performance. Effects of Met source supplementing in diet on growth performance of piglets are presented in Table 3. Piglets fed diets supplemented with DLM or LMA had significantly

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Table 3. Effects of methionine sources on production performance of nursery pigs

Item	Control	DLM	LMA	*P*-value
Body weight (kg)				
Initial	11.17 ± 0.09*	11.18 ± 0.10	11.13 ± 0.13	0.73
Final	33.50 ± 1.34^{B}	37.31 ± 1.25^{A}	37.93 ± 0.60^{A}	< 0.01
Weight gain (kg)	22.33 ± 1.35^{B}	26.13 ± 1.24^{A}	26.80 ± 0.70^{A}	< 0.01
ADG (g/day)	531.56 ± 32.24^{B}	622.13 ± 29.63^{A}	638.07 ± 16.76^{A}	< 0.01
ADFI (g/day)	1150.05 ± 54.29	1208.43 ± 11.82	1233.57 ± 66.24	0.10
FCR	2.08 ± 0.10^{b}	1.86 ± 0.08^{a}	1.85 ± 0.13^{a}	0.02
Protein intake (g/day)	230.01 ± 5.43	241.69 ± 1.18	246.71 ± 6.62	0.10

DLM = DL-methionine, LMA = liquid methionine hydroxy analog free acid, ADG = average daily gain, ADFI = average daily feed intake, FCR = feed conversion ratio

*values are means ± SD

means with different superscripts in the same row are significantly different ^{A,B}($P < 0.01$), ^{a,b}($P < 0.05$)

improved body weight ($P < 0.01$), daily weight gain ($P < 0.01$), and FCR ($P < 0.05$) compared to the piglets fed the basal diet. Feed consumption and protein intake of piglets fed the DLM or LMA diets were not different.

Blood biochemical profiles. Effects of Met sources on blood biochemical profile of piglets are presented in Table 4. The DLM or LMA supplementation significantly decreased serum urea nitrogen ($P < 0.01$). There was no significant difference among the treatments concerning blood globulin ($P > 0.05$). Both DLM and LMA supplementations significantly increased albumin in blood compared with the control group ($P < 0.01$). Supplementation with DLM resulted in a greater concentration of blood albumin than supplementation with LMA ($P < 0.01$). The albumin/globulin ratio tended to be increased when DLM was added to the basal diet ($P = 0.08$).

Dietary pH and gastrointestinal pH. The effects of Met sources on pH of the diet and gastro-

Table 4. Effect of methionine sources on blood biochemical profiles and pH in diet and gastrointestinal tract of nursery pigs

Item	Control	DLM	LMA	*P*-value
Blood biochemical profiles				
Blood urea nitrogen (mmol/l)	2.93 ± 0.21^{B*}	1.89 ± 0.08^{A}	2.00 ± 0.08^{A}	< 0.01
Total protein (g/dl)	5.82 ± 0.24	6.24 ± 0.20	6.32 ± 0.16	0.23
Albumin (g/dl)	3.89 ± 0.01^{C}	4.42 ± 0.06^{A}	4.25 ± 0.06^{B}	< 0.01
Globulin (g/dl)	2.16 ± 0.13	1.82 ± 0.19	2.07 ± 0.14	0.34
Albumin : globulin ratio	1.82 ± 0.11	2.53 ± 0.26	2.09 ± 0.15	0.08
pH in diet and gastrointestinal tract				
Diet	5.45 ± 0.02^{B*}	5.46 ± 0.02^{B}	5.36 ± 0.01^{A}	< 0.01
Stomach	3.28 ± 0.94	2.90 ± 1.19	3.88 ± 1.32	0.51
Duodenum	5.18 ± 0.42	5.43 ± 0.91	5.03 ± 0.81	0.75
Jejunum	5.93 ± 0.43	6.15 ± 0.17	6.05 ± 0.06	0.52
Ileum	6.60 ± 0.36	6.45 ± 0.06	6.95 ± 0.47	0.16
Caecum	5.88 ± 0.15	5.78 ± 0.05	5.80 ± 0.00	0.32
Colon	5.83 ± 0.13	5.80 ± 0.14	5.83 ± 0.06	0.95
Rectum	6.23 ± 0.21	6.18 ± 0.10	6.20 ± 0.18	0.92

DLM = DL-methionine, LMA = liquid methionine hydroxy analog free acid

*values are means ± SD

$^{A–C}$means with different superscripts in the same row are significantly different ($P < 0.01$)

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Table 5. Effect of methionine sources on populations of *Lactobacillus* spp. and *E. coli* in the gastrointestinal tract of nursery pigs

Item	Control	DLM	LMA	*P*-value
E. coli (log_{10}CFU/ml)				
Caecum	6.33 ± 0.92*	6.02 ± 0.58	5.68 ± 0.19	0.40
Rectum	5.96 ± 0.65	5.94 ± 0.50	5.82 ± 0.39	0.94
***Lactobacillus* spp.** (log_{10}CFU/ml)				
Caecum	8.38 ± 0.23^{A}	8.03 ± 0.18^{B}	7.86 ± 0.10^{B}	< 0.01
Rectum	8.14 ± 0.30	8.17 ± 0.02	8.09 ± 0.10	0.83

DLM = DL-methionine, LMA = liquid methionine hydroxy analog free acid

*values are means ± SD

^{A,B}means with different superscripts in the same row are significantly different ($P < 0.01$)

intestinal tract content are presented in Table 4. Dietary pH was significantly reduced due to LMA supplementation ($P < 0.01$). The pH in each segment of the digestive tract was not significantly different among treatment groups.

Population of E. coli and Lactobacillus spp. in the hindgut. Effects of Met sources on the population of *E. coli* and *Lactobacillus* spp. in the hindgut of piglets are presented in Table 5. There were no effects of supplementation of the diet with either DLM or LMA on the populations of *E. coli* in the caecum or rectum. In the case of *Lactobacillus* spp., however, the populations in the caecum were significantly decreased by both DLM and LMA supplementation ($P < 0.05$) compared with the control but no differences in the rectum were observed.

Short-chain fatty acids in the caecum. Effects of Met supplementation on concentrations of SCFAs in the caecum are shown in Table 6. Piglets fed the diet supplemented with DLM had an elevated level of succinic acid compared to those fed the LMA supplemented diet ($P < 0.05$). There were no other differences among treatments.

DISCUSSION

Growth performance. Supplementation of Met to the basal diet improved the growth performance of the piglets. The present results indicate that growth performance was promoted by both LMA and DLM supplementation. Because LMA contains 12% of water, the bioefficacy of LMA compared to DLM is 88% (weight/weight) if LMA is completely converted to Met. Several investigators have reported an 88% bioefficacy of LMA compared to DLM on equimolar basis (Chung and Baker 1992; Knight et al. 1998). In the present study, because growth performance of the LMA group was similar to that of the DLM group, it is concluded that the relative bioefficacy is equal to

Table 6. Effects of methionine sources on short chain fatty acids concentration in the caecum of nursery pigs

Item	Control	DLM	LMA	*P*-value
Total organic acids (mmol/l)	150.39 ± 13.79*	141.25 ± 20.84	138.79 ± 12.14	0.58
Formic acid (%)	2.43 ± 1.59	2.64 ± 1.50	1.16 ± 0.45	0.37
Acetic acid (%)	47.61 ± 3.44	45.81 ± 4.18	48.61 ± 2.18	0.52
Propionic acid (%)	22.44 ± 1.82	21.19 ± 4.83	23.36 ± 1.18	0.62
Butyric acid (%)	11.86 ± 2.23	10.44 ± 2.75	11.97 ± 0.60	0.53
Valeric acid (%)	5.01 ± 2.47	7.37 ± 4.64	3.76 ± 1.04	0.71
Succinic acid (%)	9.28 ± 1.36^{b}	10.91 ± 0.93^{a}	8.72 ± 0.39^{b}	0.03
Lactic acid (%)	1.96 ± 0.37	2.31 ± 1.31	2.71 ± 0.65	0.83

DLM = DL-methionine, LMA = liquid methionine hydroxy analog free acid

*values are means ± SD

^{a,b}means with different superscripts in the same row are significantly different ($P < 0.05$)

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that of DLM (88%; weight/weight). This observation is in agreement with Bunchasak (2009) who suggested that in broiler chicks, the efficacy of LMA is equal to that of DLM.

Blood biochemical profiles. Blood urea nitrogen (BUN) is commonly used as an end point for evaluation of the effect of amino acid supplementation in diets on nitrogen balance of pigs. When the limiting amino acid is supplemented, the concentration of BUN decreases continuously until the requirement for the limiting amino acid is reached (Lewis et al. 1980). In the present study, BUN was greater in the pigs fed the basal diet compared with pigs fed the Met supplemented diets. Although Met supplementations (DLM or LMA) elevated protein intake by 5.2 and 7.26% (through feed consumption), BUN dropped by 35.5 and 31.7%, respectively. This is in agreement with Feng et al. (2006) who also observed that the concentration of BUN in pigs receiving supplemental Met (DLM or LMA) was lower than that for pigs receiving a low Met-containing diet. Supplementation with Met sources improves the amino acid balance in the diet and this consequently reduces urea production from the urea cycle (Kim et al. 2006).

Albumin is commonly used as a marker in nutritional studies and directly responds to changes in both protein quantity and quality (Eggum et al. 1989). It serves as the major reservoir of protein (Margaret 2006) and is involved in colloidal osmotic pressure, acid-base balance, and it acts as a transport carrier for small molecules such as vitamins, minerals, hormones, and fatty acids (Margaret 2006). For example, albumin concentration is reduced if insufficient or excessive protein is provided and may act as a mobile source of amino acids in a nutritional emergency (Butler 1971). Supplemental Met (DLM or LMA) significantly increased serum albumin and improved growth performance which is in accordance with the results of Reifsnyder et al. (1984). Because albumin is mainly produced in the liver (globulin is produced in many sites within the body), increased serum albumin due to Met supplementation reflects an improvement of protein synthesis in the liver of pigs (Reid et al. 1968). In the present study, moreover, the increase in serum albumin in response to supplementation with DLM was significantly greater than in response to LMA which indicates that DLM may have a greater effect on protein synthesis than LMA. Using serum albumin as the criteria, therefore, the bioefficacy of LMA may be lower than 88% if compared to DLM.

Dietary pH and gastrointestinal pH. Reducing pH in the gastrointestinal tract by addition of organic acids in different combinations to weaned pig diets results in qualitative changes of microbial populations and fermentation characteristics in the small intestine and caecum (Franco et al. 2005). In the present study, it is clear that although LMA supplementation significantly reduced the pH of the diet, the acidity in each segment of the small intestine was not significantly affected and did not differ among treatment groups. This result is in accordance with the findings of Risley et al. (1992) and of Gabert and Sauer (1995) who failed to show any significant effect of organic acids on pH in small intestinal segments. The absence of any effect of pH of the diet on the pH in the various segments of the digestive tract is probably due to high degree of digestive homeostasis and differences in dietary buffering capacity (Partanen and Mroz 1999). The acid-buffering capacity was the lowest in cereals and cereal by-products, intermediate or high in protein feedstuffs, and very high in mineral sources (except in dicalcium and monosodium phosphates) (Partanen and Mroz 1999). Acid-buffering capacity of LMA (–5000 mEq/kg) or DLM (1318 mEq/kg) (Partanen and Mroz 1999; Partanen 2001; Lawlor et al. 2005) that is higher than of other common organic acids such as formic acid (–17 000 mEq/kg), and high protein (20% CP) was used in this study. This indicates that supplementing LMA in the diet could not induce high acidity in the gastrointestinal tract due to high acid-buffering capacity of the protein in the diet itself. It can be said that the significant difference in the diet pH was due to low variation among triplicate, and concluded that there was no effect of DLM and LMA supplementation on the gastrointestinal pH.

Population of E. coli and Lactobacillus spp. in the hindgut. In general, lactic acid bacteria reduce the number of pathogens and their virulence by producing antagonistic compounds, competition for adhesion sites in the mucosa, and stimulation of the host's immune response (Blomberg et al. 1993). In the present study it was observed that *Lactobacillus* spp. declined due to supplementation of DLM or LMA while growth performance was improved. Therefore, it can be concluded that performance improvement of pigs fed diets supplemented with DLM and LMA is due to correcting

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the essential amino acid imbalance in the basal diet rather than to activities of intestinal bacteria.

Short-chain fatty acids in the caecum. Acetic, propionic, and butyric acids are the main SCFAs in the hindgut of pigs (Cummings 1984; Sakata 1995). Accordingly, it was not surprising that acetic acid and propionic acid accounted for 65–69% of the total organic acids in the present study. Butyric acid is a relatively minor component compared to acetic acid and propionic acid in the lumen of the large intestine (Kameue et al. 2004), whereas it is considered to be the most effective for gut health. However, the SCFAs concentrations were not significantly different among the treatment groups. The only exception was that succinic acid level in the caecum of piglets fed DLM supplementation was higher than in pigs fed the LMA supplemented diet. The mechanism for this observation is unclear.

In summary, LMA can be used as a good source of Met (88% bioefficacy, weight/weight) in broken rice-soybean diet for improving production performance of weaned pigs such as growth rate and FCR. If serum albumin was used to evaluate the bioefficacy, the 88% bioefficacy (weight/weight) might be overestimated. Gastrointestinal pH was not affected by Met sources, but fermentation characteristics in the gastrointestinal tract may be different.

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