



## Effects of Supplemental Liquid DL-methionine Hydroxy Analog Free Acid in Diet on Growth Performance and Gastrointestinal Functions of Piglets

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**ABSTRACT:** This study was conducted to determine the effect of dietary supplementation of liquid DL-methionine hydroxy analog free acid (DL-MHA) on growth performance and gastrointestinal conditions of piglets. One hundred and eighty crossbred barrow piglets (Large White×Landrace, body weight: 12.48±0.33 kg) were divided into three groups with ten replications of six piglets each. Piglets received DL-MHA in diet at a concentration of 0 (control group), 0.15%, or 0.24%. The results indicated that increasing the standardized ileal digestible (SID) of sulfur amino acids (SAA) to lysine (SID SAA:Lys) ratio by supplementation of DL-MHA tended to increase (quadratic;  $p<0.10$ ) weight gain and ADG, and showed slightly greater (linear;  $p<0.10$ ) gain:feed ratio. The pH in the diet and cecum linearly decreased ( $p<0.01$ ), whereas pH in colon had a quadratic response ( $p<0.01$ ) with increasing supplementation of DL-MHA. By greater supplementation of DL-MHA, the population of *Lactobacillus* spp. in rectum was likely to increase (quadratic;  $p<0.10$ ), but *Escherichia coli* population in the diet was reduced (quadratic;  $p<0.05$ ). Acetic acid concentration and total short-chain fatty acids in cecum linearly increased ( $p<0.05$ ), whereas valeric acid in cecum quadratically increased ( $p<0.05$ ) with increasing DL-MHA levels. Moreover, the villous height of the jejunum quadratically increased ( $p<0.01$ ) as the supplementation of DL-MHA was increased. It is concluded that the addition of DL-MHA in diet improved the growth performance and the morphology of gastrointestinal tract of piglets. (**Key Words:** Diet, Gastrointestinal Functions, Growth Performance, Liquid DL-methionine, Piglets, Short-chain Fatty Acids)

### INTRODUCTION

Methionine (Met) is considered as the second or third limiting amino acid in diet for modern nursery pigs (Gaines et al., 2005), and it plays several roles such as an initiating amino acid in protein synthesis and as the principal biological methylating agent in the body (Bunchasak, 2014). Commercially, both in powder DL-methionine (DL-Met, 99% powder) and its analogue liquid DL-methionine hydroxy analog free acid (DL-MHA, 88% aqueous solution) are supplemented in diets or drinking water for improving growth responses (Kaewtapee et al., 2010; Krutthai et al., 2015). Although the composition or material used in diets such as practical, semi-purified, or purified diets influence the bioefficacy of DL-MHA compared to DL-Met (Bunchasak, 2014), a smaller effect of DL-MHA

and DL-Met can be seen when a practical diet formulated based on corn-soybean or broken rice-soybean is used (Bunchasak, 2014; Krutthai et al., 2015). In addition, in a broken rice-soybean based diet, Kongkaew (2014) concluded that bioefficacy of DL-MHA seemed to be 88% of DL-Met (w/w), and the standardized ileal digestible (SID) of sulfur amino acids (SAA) to Lys (SID SAA:Lys) ratio for maximum growth rate was around 0.60 to 0.63.

Liquid DL-MHA has a low pH (~1.00) due to its monocarboxylic acid with a hydroxyl group on  $\alpha$  carbon, and is defined as an organic acid (pKa: 3.86) until it is absorbed and transformed to L-Met in the liver (Dibner and Buttin, 2002). As an organic acid, DL-MHA has broader antimicrobial activities and the minimum inhibitory concentration for *Escherichia coli* (*E. coli*) was 0.24% v/v (Poosuwan et al., 2007). In piglets, *E. coli* is a major problem associated with reducing the growth performance and increasing the volume of slurry, while it is well known that an acidic condition in the intestinal tract can stimulate the growth of lactobacillus bacteria which inhibit *E. coli*

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(Risley et al., 1992), reduce the amount of digestive scouring and produce short chain fatty acids (SCFAs) along the gastrointestinal tract (Sakata et al., 1995). Consequently, DL-MHA supplementation in diet may give benefits as both a Met source and an organic acid for production performance and gut health.

Therefore, the objective of this research was to study the effect of DL-MHA supplementation in diet as a Met source and an organic acid on the growth performance, microorganism in the gastrointestinal tract and diet, SCFAs in the cecum and small intestinal morphology of piglets.

## MATERIALS AND METHODS

### Experimental animals and management

One hundred eighty 7-wk-old cross bred barrows (Large White×Landrace, 12.48±0.33 kg body weight), were housed in pens (1.00×2.50 m) and the environmental temperature was maintained at an average of 29.18±0.84°C. The duration of the experiment was 6-wk. The water was supplied to each pen by two nipples; feed and water were offered *ad libitum*. The pigs were kept, maintained and treated in accordance with International Guiding Principles for Biomedical Research Involving Animals that are accepted standards for animal welfare.

### Dietary treatments

There were three treatments with ten replications of six piglets per pen in a completely randomized design. All nutrients of basal diet were calculated to meet the recommendation of National Research Council (1998), except SAA. Since true ileal digestibility of amino acids in National Research Council (1998) was transformed to SID (Stein et al., 2007), the SID of SAA in basal diet (0.51%) was lower than the recommendation (0.58% for pigs from 10 to 20 kg). The SID of Lys, Thr, and Trp in basal diet was formulated to meet the requirements according to National Research Council (1998). Subsequently, SAA were limiting amino acids in basal diet. The optimum requirement of SID SAA:Lys ratio by supplementation with DL-MHA ranged from 60% to 62%, whereas the 69% of SID SAA:Lys ration was an excessive level, resulting in the negative effect on growth performance (Gaines et al., 2005). Furthermore, the basal diet was supplemented with DL-MHA (based on 88% bioefficacy of DL-Met; w/w; Bunchasak et al., 2014) at a concentration of 0 (basal diet), 0.15% or 0.24%, consequently the SID SAA:Lys ratio was 49%, 61%, and 69%, respectively. Accordingly, the liquid form, DL-MHA was mixed with broken rice as a carrier at ratio of 1:10, and then filtered on a screen (0.5×0.5 mm) to confirm it was well dispersed. Consequently, this product was blended with other feed ingredients. The ingredient composition of

**Table 1.** The ingredient composition of basal diet

Items	
Ingredient (%)	
Broken rice	42.97
Corn	4.99
Raw rice bran	8.13
Soybean meal (48% CP)	3.99
Full fat soybean	27.34
Fish meal (60% CP)	2.00
Soybean oil	1.29
L-Lys-HCl	0.35
L-Thr	0.10
Skim milk (33% CP)	4.99
Monocalcium phosphate (21% phosphorus)	1.82
Calcium carbonate	1.08
Salt	0.21
Premix <sup>1</sup>	0.50
Corn starch	0.24
Calculated chemical composition	
Metabolizable energy (MJ/kg) <sup>2</sup>	14.00
Crude protein (%)	20.00
Calcium (%)	1.00
Available phosphorus (%)	0.49
Standardized ileal digestible AA (%)	
Lys	1.05
Thr	0.85
Trp	0.25
Met	0.28
Cys	0.23
SAA	0.51
SAA:Lys ratio	49

CP, crude protein; AA, amino acids; SAA, sulfur amino acids.

<sup>1</sup> Premix content; Vitamin A (retinyl acetate) 1,200 mg, vitamin D<sub>3</sub> (cholecalciferol) 16,000 µg, vitamin E (dl- $\alpha$ -tocopheryl acetate) 21,818 mg, K<sub>3</sub> (menadione) 1.4 g, B<sub>1</sub> (thiamin) 0.6 g, B<sub>2</sub> (riboflavin) 0.3 g, B<sub>6</sub> (pyridoxine) 0.75 g, B<sub>12</sub> (cyanocobalamin) 14 mg, nicotinic acid 20 g, pantothenic acid 10 g, folic acid 0.44 g, D-biotin 0.04 g, choline chloride 60 g, Fe (FeSO<sub>4</sub>·H<sub>2</sub>O) 45 g, Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O) 40 g, Mn (MnO) 15 g, Zn (ZnO) 40 g, Co (CoCO<sub>3</sub>) 0.2 g, I (Ca(IO<sub>3</sub>)<sub>2</sub>) 0.4 g, Se (Na<sub>2</sub>SeO<sub>3</sub>) 0.06 g, carrier (grinded corn cob) added to 1 kg.

<sup>2</sup> The energy content was derived from the calculation.

the basal diet is shown in Table 1.

### Growth performance records

Initial body weight (d 0) and final body weight (d 41) were measured. Average daily feed intake (ADFI) was recorded daily on a per pen basis. Average daily gain (ADG) and gain:feed ratio (G:F) were calculated on a per pen basis. Due to the sparing effect between Met and Cys and that the bioefficacy of DL-MHA was 88% of DL-Met (Bunchasak, 2014), the supplemental dietary DL-MHA was considered as SAA requirement. Therefore, the SID of SAA was increased initially from 0.51% in basal diet to 0.64% which was calculated by 0.51+(0.15×0.88), and 0.72% which was

calculated by  $0.51+(0.24 \times 0.88)$  in diet supplemented with DL-MHA at 0.15% and 0.24%, respectively. Consequently, daily SAA intake was calculated from ADFI.

#### Diet and gastrointestinal pH

At the end of the trial, ten pigs per treatment were euthanized using CO<sub>2</sub> asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO<sub>2</sub>). The digesta contents from stomach, duodenum, jejunum, ileum, cecum, colon and rectum were immediately collected for the determination of pH. Moreover, diets were sampled for 10 g from each test diet and mixed with 90 mL distilled water (pH 7) for 10 min at room temperature. The pH in diet and digesta samples were directly measured with a pH meter (IQ Scientific Instruments, Inc., Carlsbad, CA, USA).

#### Bacteria counts in cecum

Five grams of digesta sample was diluted with 45 mL of 1% peptone solution (Oxoid Laboratories, Basingstoke, UK). Ten-fold serial dilution was used to reduce to 1:10 of concentration 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 agar (Difco, Becton, Dickinson and Company, Sparks, MD, USA) and MacConkey agar (Laboratorios Britania s.a., Buenos Aires, Argentina), respectively. Using the spread plate technique to determine the number of bacteria, all agar plates were incubated for 24 h at 37.0°C.

#### Analysis of short chain fatty acids

The samples of digesta from the cecum were centrifuged (TOMY model MX-301, TOMY Kogyo Co., Ltd., Tokyo, Japan) in microfuge tubes at 14,000 rpm, 4°C for 10 min, and 1.5 mL of the supernatant was transferred to a microfuge tube. The concentrations of volatile fatty acids were analyzed with gas chromatography (Shimadzu Model GC-2010 High-end, Shimadzu, Kyoto, Japan) and a flame ionization detector (GC-FID, Kyoto, Japan). One µL of the supernatant was injected into a silica capillary column (DB-WAX, 30 m × 0.25 mm i.d., film thickness of 0.50 µm, J&W Scientific, Folsom, CA, USA) at a column temperature of 150°C. The carrier gas (He) flow rate was 1.4 mL/min. and the split ratio was 1:20. The temperatures of the injection port and detector were both programmed at 225°C.

#### Morphology of small intestine

Morphology of the duodenum, jejunum and ileum was evaluated with a light microscope. The tissues were removed and immediately fixed in 10% neutral buffered formalin, and then carefully embedded in paraffin. For each paraffin block, at least 10 sections of 7 µm thickness were prepared, and then stained with hematoxylin-eosin for histological evaluation. In the morphological evaluation of

the small intestine, villous height, crypt depth and villous height: crypt depth ratio were measured. The measurement of villous height (from the tip of the villous to the villous-crypt junction) and crypt depth (from the villous-crypt junction to the lower limit of the crypt) were recorded as the mean of 10 fields for each specimen.

#### Statistical analysis

The data were analyzed as a completely randomized design using the MIXED procedures of SAS (SAS Institute Inc., 2008). The pig or pen was used as the experimental unit. Initial body weight was used as a covariate for analysis of weight gain, final weight and ADG. Orthogonal polynomial contrasts were used to assess for linear and quadratic effects of DL-MHA levels (0, 0.15%, and 0.24%). The contrast coefficients were calculated for unequally spaced treatment levels using IML procedures of SAS (SAS Institute Inc., 2008).

## RESULTS AND DISCUSSION

#### Growth performance

The results of growth performance are presented in Table 2. Increasing the SID SAA:Lys ratio by supplementation of DL-MHA tended to increase (quadratic;  $p < 0.10$ ) weight gain and ADG, and showed slightly greater (linear;  $p < 0.10$ ) G:F. There was no difference ( $p > 0.05$ ) in ADFI, but daily SAA intake was linearly increased ( $p < 0.01$ ) as DL-MHA level increased.

The supplementation of DL-MHA significantly improved the growth performance, although feed intake was depressed by about 5% and 6% in 0.15% and 0.24%, respectively. The lower feed intake may explain the decrease in growth promotion of 0.24% group. Although the relative bioefficacy of DL-MHA to DL-Met has been reported to be lower than 88% (Kim et al., 2006; Dilger and Baker, 2008), Bunchasak et al. (2014) and Krutthai et al. (2015) showed that 88% bioefficacy may be an acceptable with regard to SAA requirement. Therefore, based on 88% bioefficacy (w/w) compared to DL-Met, supplementation of DL-MHA at 0.15% and 0.24% of diet result in SID SAA:Lys ratio at 61% and 69%, respectively. This study confirms Gaines et al. (2005) and Peak (2005) who reported that optimal SID SAA:Lys ratio for modern lean-genotype piglets was approximately 60% to 62%, while an excessive Met consumption or excessively high SID SAA:Lys ratio as 0.24% DL-MHA supplementation diet (SID SAA:Lys ratio = 69%) did not give any additional benefit to the growth performance of the piglets.

#### pH in diet and gastrointestinal tract

The pH in diet and gastrointestinal tract of piglets is presented in Table 3, pH in the diet and cecum linearly

**Table 2.** Least squares means and SEM of growth performance and methionine intake of piglets receiving different amounts of DL-MHA in diet<sup>1,2</sup>

Items	Control (Basal diet)	DL-MHA 0.15%	DL-MHA 0.24%	SEM	p-values	
					Linear	Quadratic
Body weight (kg)						
d 0	12.5	12.5	12.5	0.06	NA	NA
d 41	29.0	30.7	30.0	0.41	0.064	0.051
Weight gain (kg)	16.5	18.2	17.5	0.37	0.064	0.051
ADG (g/d)	403	444	428	8.9	0.064	0.051
ADFI (g/d)	1,044	991	981	24.2	0.302	0.795
G:F	0.39	0.45	0.44	0.015	0.094	0.283
Daily SAA intake (g/d)	5.32	6.34	7.06	0.202	<0.001	0.837

SEM, standard error of the mean; DL-MHA, DL-methionine hydroxy analog free acid; NA, not statistical analysis; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain:feed ratio; SAA, sulfur amino acids.

<sup>1</sup> DL-MHA was calculated at 88% bioefficacy of DL-Met.

<sup>2</sup> Values are least squares means of 10 replicates.

decreased ( $p < 0.01$ ), whereas pH in colon had a quadratic response ( $p < 0.01$ ) with increasing supplementation of DL-MHA. However, inducing this acidity did not impact ( $p > 0.05$ ) on pH in the stomach, duodenum, jejunum and rectum, although pH in the ileum tended to linearly decrease ( $p < 0.10$ ).

Organic acids probably assist the immature digestive process of young piglets by decreasing the pH of the stomach, and promoting peptic activity and protein digestion (Kirchgessner and Roth, 1982). However, in this study, DL-MHA supplementation in diet did not affect gastric pH, which was similar to Mathew et al. (1991) and Risley et al. (1992) who reported that supplementation of organic acids in diet did not change the pH in the gut. Recently, Krutthai et al. (2015) also reported low potentiality of acidifying by DL-MHA throughout gastrointestinal tract of piglets. Since supplemental DL-MHA induces dietary acidity (Krutthai et al., 2015), the presence of an acidic diet may affect a low pH in stomach, leading to a negative feedback mechanism that inhibits HCl secretion (Yen, 2001). Moreover, an unchanged pH in some part of gastrointestinal tract (stomach, duodenum, jejunum

and ileum) may be caused by some factors such as greater feed buffer capacity (Bolduan et al., 1988), digesta transit time (Walsh et al., 2004) and physiological homeostasis in the tract.

Nevertheless, DL-MHA supplementation decreased pH in the cecum and colon where the fermentative segments are located. Generally, the retained feed residues and endogenous materials in the hind gut are fermented by microbes, and the unabsorbed DL-MHA or SAA in the hind gut (cecum and colon) may be utilized by acid bacteria to produce SCFAs or lactic acid (Macfarlane and Gibson, 1995) and result in a decrease of pH. However, the exact mechanism of this observation is unclear.

#### Microorganisms in gastrointestinal tract

The effect of supplementation of DL-MHA in diet on *Lactobacillus* spp. and *E. coli* in the hind gut of piglets is shown in Table 4. The population of *Lactobacillus* spp. in rectum was likely to increase (quadratic;  $p < 0.10$ ), but *E. coli* population in the diet was reduced (quadratic;  $p < 0.05$ ) by greater supplementation of DL-MHA.

In practice, feedstuffs and drinking water contaminated

**Table 3.** Least squares means and SEM for pH in the diet and gastrointestinal tract of piglets receiving different amounts of DL-MHA in diet<sup>1</sup>

Items	Control (Basal diet)	DL-MHA 0.15%	DL-MHA 0.24%	SEM	p-values	
					Linear	Quadratic
Diet	5.81	5.68	5.58	0.029	<0.001	0.743
Stomach	3.33	3.70	3.55	0.312	0.744	0.739
Duodenum	5.32	5.14	5.47	0.183	0.819	0.502
Jejunum	6.14	6.08	6.27	0.086	0.616	0.462
Ileum	6.70	6.57	6.41	0.067	0.087	0.718
Cecum	6.20	6.07	5.86	0.047	0.003	0.318
Colon	6.14	5.82	5.92	0.038	0.002	0.009
Rectum	6.22	6.20	6.17	0.048	0.692	0.917

SEM, standard error of the mean; DL-MHA, DL-methionine hydroxy analog free acid.

<sup>1</sup> Values are least squares means of 10 replicates.

**Table 4.** Least squares means and SEM for populations of *Lactobacillus* spp. and *E. coli* in the gastrointestinal tract and *E. coli* in diet of piglets receiving different amounts of DL-MHA (Log<sub>10</sub>cfu/mL)<sup>1</sup>

Items	Control (Basal diet)	DL-MHA 0.15%	DL-MHA 0.24%	SEM	p-values	
					Linear	Quadratic
<i>Lactobacillus</i> spp.						
Cecum	9.47	9.54	9.39	0.086	0.754	0.532
Rectum	9.46	9.62	9.37	0.057	0.700	0.085
<i>E. coli</i>						
Diet	3.97	3.50	3.66	0.090	0.019	0.027
Cecum	10.43	10.62	10.57	0.070	0.363	0.497
Rectum	9.30	9.12	9.27	0.095	0.795	0.434

SEM, standard error of the mean; DL-MHA, DL-methionine hydroxy analog free acid.

<sup>1</sup> Values are least squares means of 10 replicates.

with *E. coli* have been reported to adversely affect animal health (Risley et al., 1992). According to Mathew et al. (1991), organic acid could reduce *E. coli* populations in diet and improved growth performance. Therefore, DL-MHA may be defined as an organic acid that directly inhibits pathogenic bacteria contamination in the diet. However, DL-MHA supplementations have less effect on the populations of this microorganism in the cecum and rectum, although the pH in these segments was decreased by DL-MHA supplementation. Similarly, Risley et al. (1992) failed to depress the population of *E. coli* in the gastrointestinal tract by feeding with fumaric acid. Due to the optimal pH range of *E. coli* is 6.0 to 8.0 (Tan, 2006), it can be postulated that the reduction of pH in diets (from 6.20 to 5.86) by DL-MHA supplementation was not enough to decrease population of *E. coli* in the cecum and rectum.

#### Short chain fatty acids in cecum

The effects of the DL-MHA supplementation on the concentration of SCFAs in the cecum are presented in Table 5. The addition of DL-MHA in diet had a linear increase ( $p < 0.05$ ) in acetic acid and total SCFAs concentration in the cecum, whereas valeric acid in the cecum quadratically increased ( $p < 0.05$ ) with increasing DL-MHA levels.

Large intestine is the major site for microbial fermentation resulting in the production of gas, and the highest concentration of SCFAs is found in the cecum (Macfarlane and Gibson, 1995). In this study, an increase in

SCFAs production with supplemental dietary DL-MHA may be caused by an extension time of digestion. According to Walsh et al. (2004), a diet supplemented with organic acids had a longer retention time in stomach, thereby enhancing digestibility in piglets. Furthermore, Macfarlane and Macfarlane (1995) showed that a long transit time in the large intestine can have profound effects on bacterial physiology and metabolism, leading to protein breakdown and amino acid fermentation and making an increased contribution to colon SCFA pools.

The varieties of SCFAs in the cecum are in agreement with Wallace (1995) who reported that the concentration of acetate is greater than that of propionate and butyrate. These three SCFAs are produced from the degradation of cysteine (Cys), whereas the main products of Met degradation are propionate and butyrate (Smith and Macfarlane, 1997). Due to the sparing effect between Met and Cys, the supplementation of DL-MHA may lead quantities of residual Cys in the cecum to produce the SCFAs. Surprisingly, the amount of valeric acids, which is produced from branched-chain amino acids (BCAAs; Macfarlane and Macfarlane, 1995), was also significantly increased by DL-MHA supplementation, while the metabolic linkage between SAA and BCAAs is weak.

#### Small intestinal morphology

The effects of DL-MHA supplementation on morphology of the small intestine are shown in Table 6. The

**Table 5.** Least squares means and SEM for SCFAs concentrations in the cecum of piglets receiving different amounts of DL-MHA in diet (mmol/L)<sup>1</sup>

Items	Control (Basal diet)	DL-MHA 0.15%	DL-MHA 0.24%	SEM	p-values	
					Linear	Quadratic
Acetic acid	4.83	10.97	10.26	1.125	0.025	0.213
Propionic acid	4.03	6.86	5.92	0.636	0.163	0.223
Butyric acid	2.74	3.70	2.90	0.435	0.785	0.380
Valeric acid	0.04	0.11	0.07	0.011	0.087	0.021
Total SCFAs	11.65	22.52	22.36	2.176	0.022	0.323

SEM, standard error of the mean; SCFAs, short chain fatty acids; DL-MHA, DL-methionine hydroxy analog free acid.

<sup>1</sup> Values are least squares means of 10 replicates.

**Table 6.** Least squares means and SEM for small intestinal morphology of piglets receiving different amounts of DL-MHA in diet<sup>1</sup>

Items	Control (Basal diet)	DL-MHA 0.15%	DL-MHA 0.24%	SEM	p-values	
					Linear	Quadratic
Villous height ( $\mu\text{m}$ )						
Duodenum	438	460	435	7.6	0.995	0.137
Jejunum	376	407	369	5.3	0.893	0.002
Ileum	295	318	316	5.3	0.078	0.399
Crypt depth ( $\mu\text{m}$ )						
Duodenum	438	462	444	7.2	0.585	0.180
Jejunum	292	305	293	3.9	0.709	0.128
Ileum	322	332	291	4.9	0.025	0.005
Villous height to crypt depth ratio						
Duodenum	1.06	1.04	1.07	0.023	0.862	0.522
Jejunum	1.35	1.39	1.31	0.024	0.540	0.189
Ileum	0.97	1.01	1.17	0.023	0.001	0.104

SEM, standard error of the mean; DL-MHA, DL-methionine hydroxy analog free acid.

<sup>1</sup> Values are least squares means of 10 replicates.

villous height in segment of the jejunum had a quadratic effect ( $p < 0.01$ ), whereas villous height in segment of ileum tended to linearly increase ( $p < 0.10$ ) as supplementation of DL-MHA increased. Crypt depths in the duodenum and jejunum were not affected ( $p > 0.05$ ) by DL-MHA supplementation, whereas crypt depth in the ileum showed a quadratic effect ( $p < 0.01$ ). Subsequently, villous height to crypt depth ratio in the ileum linearly increased ( $p < 0.01$ ) with the greater supplementation of DL-MHA.

In general, SCFAs can be absorbed by diffusion in the hindgut wall as the energy substrates for mucosa development and precursors for synthesis of non-essential amino acids, DNA and greater lipids required for intestinal growth (Mroz, 2005). Furthermore, gastrointestinal cell proliferation is also stimulated by SCFAs concentration, particularly butyric acid (Sakata et al., 1995). In current study, however, a significant increase in the amount of butyric acid in the cecum was not observed, while acetic acid and valeric acid contents in the cecum were significantly increased. These findings indicated that at least acetic acid might have closely promoted physiological function of villi, although we could not find any report that indicates the effect of valerate on gastrointestinal morphology of piglets. Moreover, derivatives of DL-MHA such as taurine or glutathione which are antioxidants protect the damage of villi from oxidative stress in the small intestine (Shoveller et al., 2005) may promote the morphology.

Increasing DL-MHA increased (quadratic;  $p < 0.10$ ) growth rate, total SCFAs in cecum (linear;  $p < 0.05$ ), linearly decreased ( $p < 0.01$ ) pH in cecum, and quadratically improved ( $p < 0.01$ ) morphology of small intestine of the pigs. Therefore, it can be summarized that three mechanisms are involved in improving intestinal function by DL-MHA supplementation resulting in improvement of

growth performance as follows; i) DL-MHA directly stimulates cell elongation as a source of energy or a precursor of protein synthesis, ii) derivatives of DL-MHA such as taurine or glutathione which are antioxidants protect the damage of villi from oxidative stress in the small intestine (Lambert, 2004; Roig-Pérez et al., 2005; Shoveller et al., 2005) and iii) DL-MHA supplementation lowers pH in the cecum and the lower pH condition may increase acid bacteria and produce SCFAs to enhance the physiological status of small intestine villi by the reverse-peristalsis process.

## IMPLICATIONS

Liquid DL-MHA can be applied in diet as an acidifier since it inhibited *E. coli* contamination in diet and depressed pH in the large intestine of piglets. Therefore, the dual effect (Met source and organic acid) of 0.15% DL-MHA (61% SID SAA:Lys ratio) could promote growth performance and good conditions of the gastrointestinal tract of piglet.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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