Effects of feed grade L-methionine on intestinal redox status, intestinal development, and growth performance of young chickens compared with conventional DL-methionine

Y. B. Shen,* P. Ferket,† I. Park,* R. D. Malheiros,† and S. W. Kim*[1]

*Department of Animal Science and †Prestege Department of Poultry Science, North Carolina State University, Raleigh 27695

ABSTRACT: This study was conducted to test the effects of supplemental L-Met on redox status, gut development, and growth performance of young broiler chickens compared with DL-Met. A total of 888 (half male and half female) 1-d-old Ross 308 chickens were weighed and randomly allotted to 7 treatments in a randomized complete block design for 21 d, including a basal diet (BD), the BD + 0.095% L-Met or DL-Met, the BD + 0.190% L-Met or DL-Met, and the BD + 0.285% L-Met or DL-Met (representing 60, 70, 80, and 90% of the Met + Cys requirement). Feed disappearance and BW were recorded every 7 d. Liver and duodenum samples were collected on d 0, 7, and 21 to measure redox status and intestine morphology. On d 7, chicks fed a diet supplemented with either 0.285% L-Met or 0.285% DL-Met had increased ($P < 0.05$) concentrations of glutathione (GSH) and reduced ($P < 0.05$) protein carbonyl (PC) and malonedialdehyde contents in duodenum mucosa compared with chicks fed the BD. Chicks fed a diet supplemented with 0.285% L-Met had greater ($P < 0.05$) villus width compared with chicks fed a diet supplemented with 0.285% DL-Met. Chicks fed a diet supplemented with 0.285% L-Met had lower ($P < 0.05$) crypt depth and greater ($P < 0.05$) villus height:crypt depth ratio compared with chicks fed a diet supplemented with 0.285% DL-Met or the BD. On d 21, chicks fed a diet supplemented with 0.285% L-Met had increased ($P < 0.01$) concentrations of GSH and total antioxidant capacity (TAC) but reduced ($P < 0.05$) PC content in duodenum mucosa compared with chicks fed a diet supplemented with 0.285% DL-Met and the BD. Chicks fed a diet supplemented with 0.285% L-Met had greater ($P < 0.05$) villus height compared with chicks fed the BD. During the entire 21-d supplementation of either L-Met or DL-Met, ADG and G:F were enhanced ($P < 0.01$) compared with chicks fed the BD. Chicks fed diets supplemented with L-Met had greater ($P < 0.05$) ADG and G:F than chicks fed diets supplemented with DL-Met. The relative bioavailability of L-Met to DL-Met for ADG and G:F was 138.2 and 140.7%, respectively. Overall, supplementation of either L-Met or DL-Met has beneficial effects on villus development in association with increased GSH production and levels of TAC and reduced protein oxidation in duodenum. Supplementation of L-Met served a better function on redox status and development of the gut of chicks compared with DL-Met. Chicks fed diets with L-Met had better growth response than chicks fed diets with DL-Met.

Key words: broiler chicken, DL-methionine, growth, L-methionine

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INTRODUCTION

Methionine is an essential AA for animals. The sulfur-containing side chain makes Met a major AA not only for protein synthesis but also for other various biological functions. Previous research investigated the functional role of Met, including methyl donation (Finkelstein, 1990), antioxidative effects (Reddy et al., 1994; Luo and Levine, 2009), and a precursor of bioactive compounds such as glutathione (**GSH**) and taurine (Finkelstein, 1990). The various functional roles of Met are important for the development and health status of animals (Stoll et al., 1998; Riedijk et al., 2007; Shen et al., 2014).

[1]Corresponding author: sungwoo_kim@ncsu.edu
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For poultry, Met is the first limiting AA in corn–soybean meal–based diets (Dilger and Baker, 2007). Deficiency of Met in conventional poultry diets leads to a great demand. However, pure crystalline L-Met is not commercially available for animal production. Conventionally, in poultry diets, supplemental Met is provided as either DL-Met (99% purity power), which contains 50% L-Met and 50% D-Met, or an aqueous solution of 2-hydroxy-4-(methylthio) butanoic acid (**HMTBA**). Generally, poultry can utilize the isomers and analogs of Met for protein synthesis, because of the unique enzymatic pathways to convert Met isomers and analogs to L-Met in the liver and kidney (Baker, 2006; Thwaites and Anderson, 2007). In fact, there is a considerable volume of broiler data evaluating the relative efficacy between the isomers and analogs of Met. Studies have shown on D-Met is 90 to 100% as efficacious as L-Met (Marrett and Sunde, 1965; Bhargava et al., 1970, 1971; Sunde, 1972; Katz and Baker, 1975; Baker, 1986) whereas HMTBA is 65 to 100% as efficacious as DL-Met (Sauer et al., 2008).

However, the key enzyme, D-amino acid oxidase converting D isomer and D analog to L-Met, exists only in the liver and kidney (Bauriedel, 1963). Therefore, D-Met is not utilized directly by the cells of the gastrointestinal tract until it is converted to L-Met in either the liver or the kidneys. Research also showed that the expression of this enzyme is very low for young animals (D'Aniello et al., 1993). Therefore, L-Met is the only biologically functional form of Met readily utilized by the intestinal cells of young animals. Interestingly, Met tends to have a great rate of first pass metabolism in the gut (Stoll et al., 1998). The significant splanchnic Met metabolism indicates that the gastrointestinal tract could have a functional requirement for Met (Shoveller et al., 2003). Therefore, we hypothesize that supplementation of feed grade L-Met (99% purity powder), which is an immediate source of Met for splanchnic metabolism, would have better effects on GSH level, redox status, and, consequently, gastrointestinal tract development and growth performance of young broiler chicks compared with the use of feed grade DL-Met.

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee at North Carolina State University (Raleigh, NC).

Animal and Design

The experiment was conducted at the North Carolina State University Scott Hall (Raleigh, NC). A total of 888 (half male and half female) 1-d-old Ross 308 broiler chicks hatched from broiler breeders maintained at the Piedmont Research Station (Salisbury, NC) were used in this study. On d 0 of this experiment, 12 chicks were randomly selected and euthanized to collect duodenum and liver tissue. The rest of chicks were then weighed and randomly allotted to 7 dietary treatments in a randomized complete block design on the day of hatching. Dietary treatments included 1) a basal diet (**BD**), 2) the BD + 0.095% L-Met, 3) the BD + 0.190% L-Met, 4) the BD + 0.285% L-Met, 5) the BD + 0.095% DL-Met, 6) the BD + 0.190% DL-Met, and 7) the BD + 0.285% DL-Met. In the beginning of the study, each treatment contained 12 cages with 10 chicks per cage, except for the BD, the BD + 0.285% L-Met, and the BD + 0.285% DL-Met treatments, which had 12 cages with 11 chicks per cage. Chicks of the same gender were placed in 1 cage. The cage was considered the experimental unit. Chicks were reared in cages in 2 windowless air-conditioner controlled houses for 21 d. Each room contained four 12-cage Petersime batteries (Petersime Incubator Co., Gettysburg, OH). Out of 12 cages in each treatment, 6 cages (half male and half female) of chicks were housed in 1 room. The other 6 cages were housed in another room. Sex and house were block factors. Each cage was 0.8 m wide by 1.2 m in length (10 chicks/m^2) and contained 1 cuboid shape drinker and feeder. Chicks had ad libitum access to water and feed throughout the study. Feed additions were weighed and recorded. Feeders were shaken once per day. The broilers and feed were weighed on d 0, 7, 14, and 21 for computation of growth performance. Dead chicks were removed and weighed daily to calculate mortality and adjusted growth performance data. The lighting program started with 23 h of light from 1 to 7 d, 22 h of light to 14 d, and 20 h of light to 21 d. The temperature from hatching to 7 d was maintained at 32 to 34°C, 29°C to 14 d, and 27°C to 21 d.

The BD (Table 1) was formulated to be deficient in Met according to the Ross nutrient specification (Aviagen, 2007). The digestible Met and Met + Cys in the BD were 0.28 and 0.56%, respectively. The BD contained Met and Met + Cys at 60% of the Ross nutrient specification (Aviagen, 2007), but all other essential nutrients were adequate. The dietary treatments supplemented with increasing levels of either L-Met or DL-Met brought the Met content to 70, 80, or 90% of the Met + Cys requirement (Aviagen, 2007). Supplemental L-Met was obtained from a commercial company (CJ CheilJedang Co., Seoul, Korea), whereas DL-Met was commercially available and purchased locally by North Carolina State University Feed Mill (Raleigh, NC). Analyses verified that L-Met and DL-Met used in this

study contained 99.1 and 99.2% active substance, respectively. Test diets were prepared by adding the different sources of Met to a single common batch of the BD to minimize unintended variations. The concentrations of AA in the final test diets were confirmed by HPLC involving precolumn derivatization with *o*-phthaldialdehyde as described by Mateo et al. (2007).

Sample Collection and Processing

On d 0, 12 chicks were randomly selected and euthanized. Then, on d 7 and 21, 1 bird representing the average weight of each cage was selected and euthanized from the BD, the BD + 0.285% L-Met, and the BD + 0.285% DL-Met treatments. After euthanasia, the gastrointestinal tract and liver were quickly dissected. The middle section of the duodenum was isolated and flushed with saline solution. Half of the duodenum was fixed in 10% formaldehyde–phosphate buffer and kept for microscopic assessment of mucosal morphology (Shen et al., 2009). The other half of the duodenum was then opened for scraping the mucosa layer of the intestine. The mucosa of duodenum was scraped into a microassay tube and frozen in liquid nitrogen. On d 21, a part of the liver was also collected into a microassay tube and frozen in liquid nitrogen. Mucosa and liver samples were then stored at –80°C until analyzed for concentrations of GSH, total antioxidant capacity (**TAC**), protein carbonyl (**PC**), and malonedialdehyde (**MDA**) as markers for oxidative and antioxidative status.

Glutathione

Glutathione is a ubiquitous antioxidant in cells. Mucosa samples (200 mg) of duodenum and liver (200 mg) were weighed and suspended into 0.6 mL ice-cold buffer containing 5% metaphosphoric acid (Shen et al., 2014). Samples were homogenized using a glass pestle on ice. The homogenate was centrifuged at 15,000 × *g* at 4°C for 30 min. The supernatant was used to determine concentrations of total GSH using an ELISA kit (STA-312; Cell Biolabs, San Diego, CA) and protein concentrations using a commercial kit (Thermo Fisher Scientific Inc., Rockford, IL; Shen et al., 2012a). Concentrations of total GSH were expressed as micromoles/gram protein.

Table 1. Composition of the basal diet (%, as-fed basis)[1]

Ingredient	%
Corn	58.65
Soybean meal	34.34
L-Lys HCl	0.28
L-Thr	0.11
L-Cys	0.03
Dicalcium phosphate	2.16
Ground limestone	1.28
Salt	0.40
Vitamin premix[2]	0.05
Mineral premix[3]	0.20
Sodium selenite premix	0.05
Choline chloride (60% choline)	0.20
Poultry fat	2.25
Total	100.00
Calculated nutrient composition	
DM, %	91.4
ME, Mcal/kg	3.05
CP, %	21.24
Crude fat, %	4.34
Digestible Arg, %	1.30
Digestible Lys, %	1.27
Digestible Met, %	0.28 (0.23)[4]
Digestible Met + Cys, %	0.56 (0.49)[4]
Digestible Thr, %	0.83
Digestible Trp, %	0.22
Digestible Leu, %	1.63
Digestible Ile, %	0.83
Digestible Val, %	0.90
Ca, %	1.05
Available P, %	0.50
Total P, %	0.75

[1]The basal diet contained 0.28 and 0.28% digestible Met and Cys, respectively, which represented 60% of the total sulfur AA requirement of a broiler. The dietary treatments supplemented with increasing levels (0.095, 0.190, or 0.285%) of either DL-Met or L-Met brought the total sulfur AA content to 70, 80, and 90% of total sulfur AA requirement.

[2]Vitamin premix (NCSU-90; North Carolina State University, Raleigh, NC) provided per kilogram of diet: 6,600 IU vitamin A, 2,000 IU cholecalciferol, 33 IU vitamin E (spring time study), 19.8 μg vitamin B_{12}, 6.6 mg riboflavin, 55 mg niacin, 11 mg pantothenic acid, 2 mg vitamin K, 1.1 mg folic acid, 2 mg thiamine, 4 mg pyridoxine, and 126 μg biotin.

[3]Trace mineral (TM-90; North Carolina State University, Raleigh, NC) premix provided in milligrams per kilogram of diet: Mn as manganous oxide, 120; Zn as zinc sulfate, 120; Fe as ferrous sulfate, 80; Cu as copper sulfate, 10; I as ethylenediamine dihydroiodide, 2.5; and Co as cobalt-iodized salt, 1.0. Selenium premix as sodium selenite was provided to each diet at a level to assure a concentration of 0.1 mg/kg.

[4]Analyzed total AA value in a parenthesis.

Total Antioxidant Capacity

Total antioxidant capacity characterizes the capacity of cells to deal with reactive oxygen species and free radicals. Mucosa samples (200 mg) of the duodenum and liver (200 mg) were weighed, suspended into 0.6 mL PBS buffer, and homogenized (Tissuemiser; Thermo Fisher Scientific Inc.) on ice. The homogenate was centrifuged at 15,000 × *g* at 4°C for 30 min. The supernatant was used to determine concentrations of TAC using an ELISA kit (STA-360; Cell Biolabs) and protein concentrations using a commercial kit (Thermo Fisher Scientific Inc.; Shen et al., 2012a). Concentrations of TAC were expressed as micromoles/gram protein.

Malonedialdehyde

Concentrations of MDA in mucosa of the duodenum and liver, as an index of lipid peroxidation, were analyzed using an ELISA kit (STA-330; Cell Biolabs) as described by Zhao et al. (2013). Mucosa samples (150 mg) of the duodenum and liver (150 mg) were weighed and suspended into 0.6 mL PBS containing 0.05% butylated hydroxytoluene. The homogenate was prepared as mentioned in the TAC analysis. The supernatant was used to determine concentrations of MDA and protein concentrations. Protein concentrations were determined using a commercial kit (Thermo Fisher Scientific Inc.; Shen et al., 2012b). Concentrations of MDA were expressed as micromoles/gram protein.

Assessment of Protein Oxidation

Protein carbonyl is a biomarker of oxidative stress. Among the biomarkers of oxidative stress, PC has some advantages because of the relative early formation and the relative stability of carbonylated proteins. For the assessment of PC content, mucosa samples (150 mg) of the duodenum were again weighed, suspended into 0.6 mL PBS buffer, and homogenized on ice (Zhao et al., 2013). The homogenate was prepared as mentioned in the TAC analysis. The supernatant was used to determine concentrations of PC using an ELISA kit (STA-310; Cell Biolabs) and protein concentrations using a commercial kit (Thermo Fisher Scientific Inc.; Shen et al., 2012b). Concentrations of PC were expressed as micromoles/gram protein.

Small Intestinal Morphology

The segments of the duodenum were embedded in paraffin, cut across the section to 5-mM-thick slides, and mounted on a polylysine-coated slide. Then, slides were stained (with hematoxylin and eosin) and examined under a Sony CCD color video camera attached to an Olympus Van-Ox S microscope (Opelco, Washington, DC). Villus height (from the tip of the villi to the villous–crypt junction), villus width (width of the villus at one-half of the villus height), and crypt depth (from this junction to the base of the crypt) were determined (Shen et al., 2009). Lengths of 10 well-oriented intact villi and their associated crypt were measured in each slide. The same person executed all the analysis of intestinal morphology.

Statistical Analysis

Data for each response were analyzed using Mixed Model (PROC MIXED) of SAS (SAS Inst. Inc., Cary, NC). The design was a randomized complete block design. The cage was considered the experimental unit. Sex and houses were block factors. For growth performance, preplanned contrasts were used to evaluate the effects of methionine sources (the BD vs. the average of 3 supplemental levels of L-Met [**LMET**], the BD vs. the average of 3 supplement levels of DL-Met [**DLM**], and LMET vs. DLM). A mutilinear regression analysis was used to evaluate the relative bioavailability (**RBA**) of L-Met to DL-Met (Littell et al., 1997; Kim and Easter, 2001; Ji et al., 2006). The following mutilinear regression was applied:

$$y = a + (b_1x_1 + b_2x_2),$$

in which y = growth performance criterion (weight gain and G:F), a = intercept (growth performance achieved with the BD), b_1 = the slope of LMET line, b_2 = the slope of DLM line, x_1 = intake of supplemental of L-Met, and x_2 = intake of supplemental of DL-Met. The RBA values of LMET to DLM were given by the ratio of the slope coefficients, b_1:b_2, according to Littell et al. (1997). For other physiological changes, statistical differences among treatments were determined by the PDIFF option of the LSMEANS statement in GLM procedure. Statistical differences were considered significant with $P < 0.05$, whereas $0.05 < P < 0.10$ was used as the criteria for tendency.

RESULTS

Glutathione

Concentrations of GSH in duodenum mucosa were measured over time (Table 2). On d 7, chicks fed a diet supplemented with either 0.285% L-Met or 0.285% DL-Met had greater ($P < 0.05$) concentrations of GSH in duodenum mucosa compared with chicks fed the BD. There were no differences between supplementation of 0.285% L-Met and 0.285% DL-Met on concentrations of GSH in duodenum mucosa. On d 21, chicks fed a diet supplemented with 0.285% L-Met had greater ($P < 0.001$) concentrations of GSH in duodenum mucosa compared with chicks fed the BD and chicks fed a diet supplemented with 0.285% DL-Met.

Concentrations of GSH were measured in liver on d 21 (Table 3). Chicks fed a diet supplemented with either 0.285% L-Met or 0.285% DL-Met had greater ($P < 0.05$) concentrations of GSH in liver compared with chicks fed the BD. There was no difference between supplementation of 0.285% L-Met and 0.285% DL-Met on concentrations of GSH in liver.

Total Antioxidant Capacity

Levels of TAC in duodenum mucosa were measured over time (Table 2). On d 7, chicks fed a diet

Table 2. Effect of supplementation of DL-Met and L-Met on duodenum redox status of broilers[1]

Item[2]	BD[3]	DLM[4]	LMET[5]	SEM	*P*-value
GSH, μmol/g protein					
d 0	14.8	14.8	14.8		
d 7	26.0[a]	45.1[b]	56.5[b]	8.5	0.050
d 21	34.5[a]	42.7[a]	63.0[b]	6.3	0.010
TAC, μmol/g protein					
d 0	139	139	139		
d 7	156[a]	164[ab]	174[b]	5	0.032
d 21	195[a]	201[a]	214[b]	5	0.018
PC, μmol/g protein					
d 0	1.50	1.50	1.50		
d 7	1.98[a]	1.55[b]	1.43[b]	0.15	0.032
d 21	2.12[a]	2.40[b]	2.08[a]	0.09	0.040
MDA, μmol/g protein					
d 0	0.54	0.54	0.54		
d 7	0.51[a]	0.37[b]	0.35[b]	0.04	0.031
d 21	0.27	0.23	0.18	0.03	0.106

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Each mean represents 12 chicks of 12 cages.

[2]GSH = glutathione; TAC = total antioxidant capacity; PC = protein carbonyl; MDA = malonedialdehyde.

[3]BD = basal diet.

[4]DLM = average of 3 supplement levels of DL-Met.

[5]LMET = average of 3 supplemental levels of L-Met.

supplemented with 0.285% L-Met had greater ($P < 0.05$) TAC in duodenum mucosa compared with chicks fed the BD. There were no difference between supplementation of 0.285% L-Met and 0.285% DL-Met on level of TAC in duodenum mucosa. On d 21, chicks fed a diet supplemented with 0.285% L-Met had a greater ($P < 0.05$) level of TAC in duodenum mucosa compared with chicks fed the BD and chicks fed a diet supplemented with 0.285% DL-Met.

Levels of TAC were measured in the liver on d 21 (Table 3). Chicks fed a diet supplemented with either 0.285% L-Met or 0.285% DL-Met had less ($P < 0.05$) TAC in the liver compared with chicks fed the BD. Chicks fed a diet supplemented with 0.285% L-Met had a greater ($P < 0.05$) level of TAC in the liver than chicks fed a diet supplemented with 0.285% DL-Met.

Protein Carbonyl

Concentrations of PC in duodenum mucosa were measured over time (Table 2). On d 7, chicks fed a diet supplemented with either 0.285% L-Met or 0.285% DL-Met had less ($P < 0.05$) PC content in duodenum mucosa compared with chicks fed the BD. There was no difference between supplementation of 0.285% L-Met and 0.285% DL-Met on PC content in duodenum mucosa. On d 21, chicks fed a diet supplemented with 0.285% L-Met and the BD had less ($P < 0.05$) PC content in duodenum mucosa compared with chicks fed a diet supplemented with 0.285% DL-Met.

Table 3. Effect of supplementation of DL-Met and L-Met on liver redox status of broilers[1]

Item[2]	BD[3]	DLM[4]	LMET[5]	SEM	*P*-value
GSH, μmol/g protein	1,093[a]	1,513[b]	1,628[b]	146	0.035
TAC, μmol/g protein	1.06[a]	0.90[c]	0.99[b]	0.02	<0.001
PC, μmol/g protein	0.94[a]	1.74[c]	1.42[b]	0.10	<0.001
MDA, μmol/g protein	18.7	16.5	17.1	1.3	0.480

[a–c]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Each mean represents 12 chicks of 12 cages.

[2]GSH = glutathione; TAC = total antioxidant capacity; PC = protein carbonyl; MDA = malonedialdehyde.

[3]BD = basal diet.

[4]DLM = average of 3 supplement levels of DL-Met.

[5]LMET = average of 3 supplemental levels of L-Met.

Concentrations of PC were measured in the liver on d 21 (Table 3). Chicks fed a diet supplemented with either 0.285% L-Met or 0.285% DL-Met had more ($P < 0.05$) PC content in the liver compared with chicks fed the BD. Chicks fed a diet supplemented with 0.285% L-Met had less ($P < 0.05$) PC content in the liver than chicks fed a diet supplemented with 0.285% DL-Met.

Malonedialdehyde

Concentrations of MDA in duodenum mucosa were measured over time (Table 2). On d 7, chicks fed a diet supplemented with either 0.285% L-Met or 0.285% DL-Met had less ($P < 0.05$) concentrations of MDA in duodenum mucosa compared with chicks fed the BD. There were no differences between supplementation of 0.285% L-Met and 0.285% DL-Met on concentration of MDA in duodenum mucosa. On d 21, there was no difference among treatments on concentration of MDA. Concentrations of MDA were measured in the liver on d 21 (Table 3). There was no difference among treatments on concentration of MDA in the liver.

Small Intestinal Morphology

Histology of duodenum was measured over time (Table 4). On d 7, chicks fed a diet supplemented with 0.285% L-Met or the BD had greater ($P < 0.05$) villus width compared with chicks fed a diet supplemented with 0.285% DL-Met. Chicks fed a diet supplemented with 0.285% L-Met had lower ($P < 0.05$) crypt depth compared with chicks fed a diet supplemented with 0.285% DL-Met or chicks fed the BD. Chicks fed a diet supplemented with 0.285% L-Met had greater ($P < 0.05$) villus height:crypt depth ratio compared with chicks fed a diet supplemented with 0.285% DL-Met or chicks fed the BD. On d 21, chicks fed a diet

Table 4. Effect of supplementation of DL-Met and L-Met on duodenum morphology of broilers[1]

Item	BD[2]	DLM[3]	LMET[4]	SEM	*P*-value
Villus height, μm					
d 0	656	656	656		
d 7	1,286	1,320	1,371	32	0.172
d 21	1,886[a]	1,957[ab]	2,038[b]	48	0.093
Villus width, μm					
d 0	94	94	94		
d 7	94[a]	85[b]	96[a]	3	0.058
d 21	119[a]	149[b]	160[b]	6	<0.001
Crypt depth, μm					
d 0	101	101	101		
d 7	171[a]	167[a]	150[b]	6	0.041
d 21	211	219	209	7	0.608
Villus height:crypt depth					
d 0	6.64	6.64	6.64		
d 7	7.69[a]	8.01[a]	9.15[b]	0.34	0.012
d 21	9.05	9.11	9.83	0.38	0.295

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Each mean represents 12 chicks of 12 cages.

[2]BD = basal diet.

[3]DLM = average of 3 supplement levels of DL-Met.

[4]LMET = average of 3 supplemental levels of L-Met.

supplemented with 0.285% L-Met had greater ($P < 0.05$) villus height compared with chicks fed the BD. Chicks fed a diet supplemented with either 0.285% L-Met or 0.285% DL-Met had greater ($P < 0.05$) villus width compared with chicks fed the BD.

Growth Performance

During the first 7 d, supplementation of L-Met increased ($P < 0.05$) ADG, ADFI, and G:F compared with chicks fed the BD (Table 5). Chicks fed diets supplemented with L-Met had greater ($P < 0.05$) ADG and tended to have greater ($P = 0.061$) G:F than chicks fed diets supplemented with DL-Met. During the entire 21 d, supplementation of either L-Met or DL-Met enhanced ADG and G:F ($P < 0.001$) compared with chicks fed the BD. Chicks fed diets supplemented with L-Met had greater ($P < 0.05$) ADG and G:F than chicks fed diets supplemented with DL-Met.

For determination of the RBA of L-Met to DL-Met, the comparison of mutilinear regression equations for LMET and DLM was applied in this study. Different statistical models can be used to determine the RBA such as multilinear regression equations (Kim and Easter, 2001; Ji et al., 2006) or nonlinear exponential regression (Lemme et al., 2002; Shen et al., 2014). In this study, multilinear regression equations were used because they provided the best model to fit the observations. In the mutilinear regression analysis, the absolute daily intake of either supplemental L-Met or DL-Met was used for the *x* axis. During the first 7 d, the RBA of L-Met to DL-Met for ADG and G:F was 217.2 and 436.9%, respectively (Fig. 1 and 2). During the entire 21 d, the RBA of L-Met to DL-Met for ADG and G:F was 138.2 and 140.7%, respectively (Fig. 3 and 4).

Table 5. Growth performance of broilers fed graded levels of either DL-Met or L-Met[1]

		Added DL-Met, % (DLM[3])			Added L-Met, % (LMET[4])				*P*-value		
Item	BD[2]	0.095	0.190	0.285	0.095	0.190	0.285	SEM	BD vs. DLM	BD vs. LMET	LMET vs. DLM
d 0 to 7											
ADG, g	12.7	13.2	13.2	13.6	13.8	14.5	13.6	0.4	0.194	0.007	0.040
ADFI, g	17.2	17.4	17.6	18.0	18.2	18.5	17.4	0.3	0.164	0.025	0.212
G:F	0.740	0.755	0.749	0.755	0.761	0.785	0.785	0.015	0.440	0.037	0.061
d 7 to 14											
ADG, g	31.3	33.7	33.1	34.6	35.1	35.8	33.4	1.0	0.030	0.004	0.263
ADFI, g	48.5	46.5	45.4	46.9	47.6	48.1	44.5	0.9	0.051	0.130	0.522
G:F	0.647	0.724	0.729	0.738	0.737	0.743	0.749	0.014	<0.001	<0.001	0.290
d 14 to 21											
ADG, g	48.1	53.4	55.0	56.8	55.1	58.7	58.3	1.8	0.001	<0.001	0.131
ADFI, g	80.5	76.8	77.8	80.1	78.9	80.4	78.4	1.8	0.274	0.531	0.505
G:F	0.598	0.694	0.706	0.709	0.698	0.730	0.746	0.017	<0.001	<0.001	0.128
d 0 to 21											
ADG, g	30.7	33.5	33.8	35.0	34.7	36.3	35.1	0.8	<0.001	<0.001	0.054
ADFI, g	48.7	46.9	47.0	48.3	48.2	49.0	46.8	0.9	0.219	0.505	0.423
G:F	0.631	0.712	0.719	0.724	0.719	0.741	0.752	0.010	<0.001	<0.001	0.022

[1]Each mean represents 12 cages of 10 chicks per pen. No room or sex effects were detected on growth performance.

[2]BD = basal diet.

[3]DLM = average of 3 supplement levels of DL-Met.

[4]LMET = average of 3 supplemental levels of L-Met.

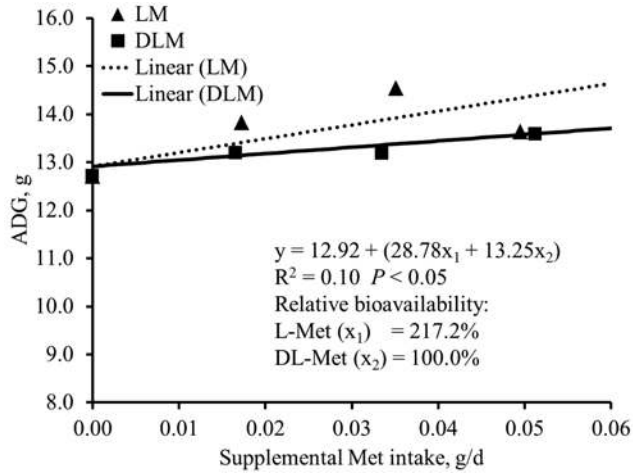

Figure 1. Daily gain of broilers with increasing intake levels of either supplemental L-Met (LM) or DL-Met (DLM) from d 0 to 7.

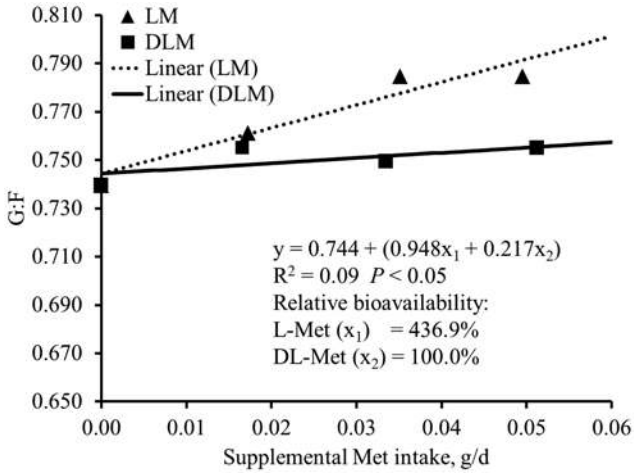

Figure 2. Gain:feed ratio of broilers with increasing intake levels of either supplemental L-Met (LM) or DL-Met (DLM) from d 0 to 7.

DISSCUSSION

The growth and development of the gastrointestinal tract requires a variety of functions of AA metabolism, including protein synthesis, cell signaling, antioxidative function, and immune function (Shoveller et al., 2003; Wang et al., 2009). Studies have shown that one-third of dietary intake of essential AA is removed in first pass metabolism by the intestine (Stoll et al., 1998). Metabolism of essential AA by the mucosal cells is quantitatively greater than AA incorporation into mucosal protein (Stoll et al., 1998). So it has been proposed that the metabolism and functionalities of AA may represent a functional requirement by the gastrointestinal tract (Windmueller and Spaeth, 1980; Roberton et al., 1991; Stoll et al., 1998; Riedijk et al., 2007). Notably, when comparing the essential AA that are being metabolized in gastrointestinal tract, on average, the utilization of Met tends to be greater than other essential AA (Stoll et al., 1998). Therefore, there appears to be a specific functional need for Met in the gastrointestinal tract of animals.

Methionine is an important methyl donor for most biological methylation reactions (Brosnan and Brosnan, 2006). Methionine is also a precursor for Cys, which plays a key role in maintaining protein function and redox status. In addition, Met serves as an indirect precursor of GSH (through Cys), taurine, and inorganic sulfur, which are also major cellular antioxidants (Brosnan and Brosnan, 2006). Therefore, the functional role of Met in the gastrointestinal tract, especially its antioxidative effect, may be the key effects on the health of the gastrointestinal tract of a rapid growing animal and consequently impact its growth (Shen et al., 2014). In the current study, supplementation of either form of Met increased levels of total GSH and TAC, reduced concentrations of PC and MDA in duodenum mucosa, and improved villus development. Those results indicated that the beneficial effects of Met on the growth and

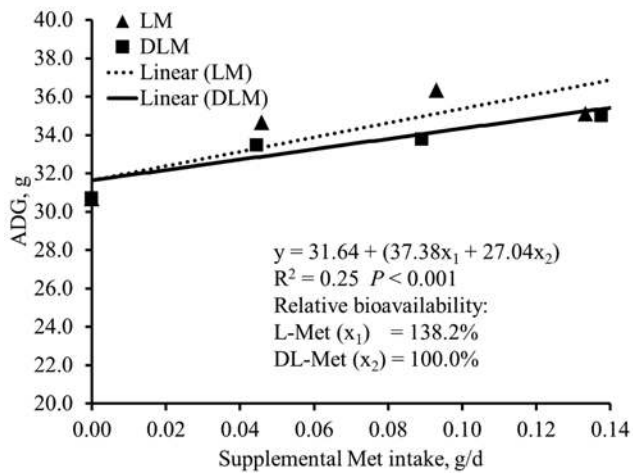

Figure 3. Daily gain of broilers with increasing intake levels of either supplemental L-Met (LM) or DL-Met (DLM) from d 0 to 21.

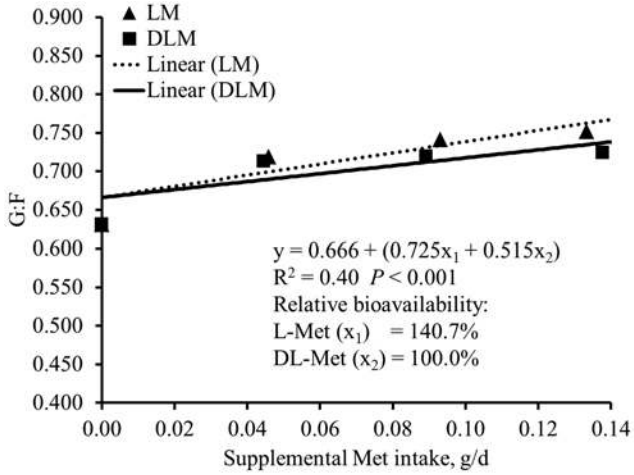

Figure 4. Gain:feed ratio of broilers with increasing intake levels of either supplemental L-Met (LM) or DL-Met (DLM) from d 0 to 21.

development of the gastrointestinal tract might be attributed to its antioxidative function.

Notably, the levels of GSH and TAC in duodenum mucosa were increased for chicks fed a diet supplemented with 0.285% L-Met compared with chicks with 0.285% DL-Met. Levels of PC in duodenum mucosa, which are indicators of protein oxidation, were reduced for chicks fed a diet supplemented with 0.285% L-Met compared with chicks with 0.285% DL-Met. These results indicated L-Met serves a better role in improving the redox statue of gastric intestinal mucosa cell compared with DL-Met. Our data also showed that chicks fed a diet supplemented with 0.285% L-Met had better villus height:crypt depth ratio compared with chicks fed with 0.285% DL-Met. The redox and morphology data supported the hypothesis that L-Met as a direct source of Met had greater beneficial effects on gastrointestinal tract development compared with DL-Met.

One possible explanation for the better antioxidative effect of L-Met compared with DL-Met could be the direct conversion to Cys and synthesis of GSH in gastrointestinal tract. Glutathione plays an important role in antioxidative defense. Besides, L-Met itself is an efficient reactive oxygen species scavenger and serves as an important antioxidant under physiological conditions (Levine et al., 1996; Luo and Levine, 2009). A variety of reactive oxygen species react readily with L-Met residues in proteins to form methionine sulfoxide (Moskovitz et al., 1997, 2001; Kryukov et al., 2002). Then, methionine sulfoxide reductases catalyze a reduction of methionine sulfoxide back to L-Met, consequently scavenging the reactive species (Kryukov et al., 2002; Luo and Levine, 2009). D-Methionine can be oxidized to D-methionine sulfoxide. However, a study has shown D-methionine sulfoxide is not sensitive to methionine sulfoxide reductases and could not be reduced back to D-Met. Consequently, D-Met has less antioxiditive function in mucosa cell compared with L-Met (Kuzmicky et al., 1977; Friedman and Gumbmann, 1988). Morphology data showed a reduced villus width in the DLM group on d 7. These data indicated that D-Met and its metabolite D-methionine sulfoxide might be slightly toxic when chicks had limited ability to convert D-Met to L-Met (Baker and Boebel, 1980).

In the liver, supplementation 0.285% of either L-Met or DL-Met increased total GSH production. This confirmed that supplementation of either form of Met improved the metabolic function of Met. Interestingly, chicks fed the BD had higher levels of TAC and lower levels of PC as well as MDA compared with chicks fed diets supplemented with crystal Met. Notably, the BD is imbalanced in AA. Amino acid imbalance would result in increased uric acid production in the liver of chicks (Donsbough et al., 2010). Uric acid is an effective antioxidant (Ames et al., 1981). Therefore, it is proposed that increased levels of TAC and reduced oxidative stress in liver of chicks fed the BD is due to the overproduction of uric acid.

One of the objectives of the current study was to compare the growth response of young broiler chicks fed diets supplemented with either L-Met or DL-Met, confirming that L-Met is better utilized for intestinal development and, consequently, growth by young broiler chicks compared with D-Met. The RBA of L-Met to DL-Met was calculated as 138 and 141% for the overall ADG and G:F, respectively. These results indicate that chicks required 138 or 141 units of DL-Met to achieve the overall ADG and G:F that were produced by 100 units of L-Met. Our results supported the hypothesis that L-Met is better utilized by young broiler chicks compared with D-Met. The difference in the functionalities and roles for intestinal development of young broiler chicks are speculated as the reasons for the difference in growth performance. This finding largely agrees with Marrett and Sunde (1965) and Bhargava et al. (1970), who indicate that chicks fed with L-Met grow better than D-Met. Bhargava et al. (1971) also showed the improved growth by using L-Met associated with increased antibodies production.

Despite our clear results, not all studies have shown consistent responses between D-Met and L-Met. Several studies indicate chicks can use DL-Met with the same efficacy as L-Met (Baker, 1986; Dilger and Baker, 2007). Dilger and Baker (2007) reported that the effectiveness of DL-Met to support weight gain and G:F was similar to that of L-Met in chicks. The inconstant utilization efficiency of Met isomers among studies can partly be due to differences in the ages of animals (Cho et al., 1980), because research has shown that the expression of D-amino acid oxidase is very low in young animals (D'Aniello et al., 1993). Most of the studies showing similar efficacy between L-Met and DL-Met were conducted with chicks at d 8 to 20 of age or even older (Dilger and Baker, 2007). In the current study, broiler chicks were on the dietary treatments from d 1 of age. Interestingly, the RBA of L-Met to DL-Met calculated from the growth response curves was higher for the data obtained from the first 10 d compared with the overall period. These results indicated that utilization of Met isomers may be a function of age (Shen et al., 2014).

Overall, supplementations of either L-Met or DL-Met have beneficial effects on villus development in association with increased GSH production and levels of TAC and reduced protein oxidation in duodenum. Supplementation of L-Met served a better function on redox status and development of the gut of young chicks compared with DL-Met. Chicks fed diets supplemented with L-Met had better growth response than chicks fed diets with DL-Met.

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