Effects of L-methionine on growth performance, carcass quality, feather traits, and small intestinal morphology of Pekin ducks compared with conventional DL-methionine

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ABSTRACT The research studied the effects of L-methionine (L-Met) on growth performance, carcass quality, feather traits, and small intestinal morphology of Pekin ducks compared with conventional DL-methionine (DL-Met). A total of 1080, 1-day-old male Pekin ducks were randomly allotted to 9 groups with 6 replicate pens of 20 birds each. During the starter phase (1 to 14 d), ducks were fed a basal diet (Met, 0.30%) or that supplemented with DL-Met or L-Met at 0.05, 0.10, 0.15, or 0.20% of feed. During the grower phase (15 to 35 d), ducks were fed a basal diet (Met. 0.24%) or that supplemented with DL-Met or L-Met at 0.04, 0.08, 0.12, or 0.16% of feed. Compared with ducks fed the basal diet, supplementation with either DL-Met or L-Met increased the body weight (**BW**) of ducks at days 14 and 35, increased average daily gain (ADG) and average daily feed intake (ADFI), decreased F:G at the starter phase, and increased ADG over the whole 35-d period (P < 0.05). The efficacy of L-Met compared

to DL-Met was 140.1% for 14-d BW, 137.6% for ADG and 121.0% for F:G for days 1 to 14. Ducks fed diets supplemented with L-Met had greater proportion of leg muscle, higher than in ducks provided with DL-Met (P < 0.05). The breast muscle proportion was enhanced with DL-Met rather than L-Met supplementation (P <0.01). The back feathers score and fourth primary wing feather length were increased with DL-Met or L-Met supplementation (P < 0.01), and there was increased efficacy of L-Met relative to DL-Met for back feathers score (153.1%). Dietary DL-Met or L-Met supplementation increased villus height of ileal mucosa of ducks at days 14 and 35 (P < 0.01). Overall, dietary L-Met or DL-Met supplementation affected the growth performance of ducks during the starter phase, and improved the feather traits and small intestinal morphology. The efficacy of L-Met to DL-Met ranged from 120 to 140% for growth performance of young ducks (1 to 14 d) and was 153% for the feather traits of ducks (35 d).

Key words: DL- and L-methionine, feather trait, growth performance, Pekin ducks

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INTRODUCTION

Methionine (Met) is the first limiting amino acid in corn-soybean based diets for poultry and plays vital roles for growth of chicks and ducks (Xie et al., 2004; Xie et al., 2006; Dilger and Baker, 2007). Supplemental Met is commonly used in conventional poultry diets, and it is generally provided as either DL-Met (99% purity), an aqueous solution of the Met precursor 2hydroxy-4-(methylthio) butanoic acid (Lemme et al., 2002; Mandal et al., 2004; Yi et al., 2006), or L-Met, which has recently received more attention as it became available from a fermentation process (Georgiev et al., 2002). The DL-Met commonly used is a racemic mixture of D- and L-Met, but only L-Met can be incorporated into naturally occurring proteins; D-Met must be converted to L-Met prior to protein synthesis through 2step enzymatic process (Chung and Baker, 1992). The efficacy of L-Met in comparison to DL-Met has been examined recently in pigs (Shen et al., 2014; Kong et al., 2016), broilers chicks (Shen et al., 2015), turkey poults (Park et al., 2018), and fish (Powell et al., 2017), and most of these results show slight advantages of L-Met compared with DL-Met, but with some inconsistencies; these may arise from differences in species, age, and the response variables examined.

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In meat ducks, supplementing Met to the basal diet (**BD**) affected performance and carcass quality, and optimized dietary levels maximized weight gain, feed efficiency and breast muscle yield (Xie et al., 2004; Xie et al., 2006), whereas excessive Met supplementation was toxic and depressed growth (Xie et al., 2007; Xue et al., 2018). Kluge et al. (2016) found that DLmethionine hydroxy analogue-free acid and DL-Met had similar efficacy as sources of Met for ducks during the first 3 wk of life. In addition, there was similar effectiveness between L-Met and DL-Met for growth performance in broiler chickens (Dilger and Baker, 2007; Dilger et al., 2007). Little is known about the efficacy of L-Met compared with DL-Met in meat ducks. Feathering is important in meat ducks because optimum feathering is crucial for high standards of carcass quality (Lopez-Coello, 2003) and dietary Met supplementation affects the feather traits in meat ducks with increased total and relative weight, and feather coverage (Guo, 2011; Zeng et al., 2015). To date, the impact of L-Met supplementation on feather traits, and its effect compared to DL-Met, is still unknown.

Therefore, the purpose of the present study was to examine the effects of L-methionine on growth performance, carcass quality, feather traits, and small intestinal morphology of meat ducks compared with conventional DL-methionine.

MATERIALS AND METHODS

Experimental Design and Diets

This study followed the guidelines of and was approved by the Animal Care and Use Committee of the Guangdong Academy of Agricultural Sciences. A total of 1080, 1-day-old Pekin male ducks (Guiliu Poultry Co., Ltd. Foshan, China) were randomly allotted to 9 groups, each with 6 replicate pens of 20 birds. Ducks were reared in plastic wire pens (length 350 cm \times width 250 cm). The trial experiment lasted for 35 d consisting of the starter (1 to 14 d) and grower (15 to 35 d) phases. During the starter phase, ducks were fed a BD (containing 0.30% Met), or that supplemented with DL-Met (99%; Adisseo, Guadalajara, Mexico) or L-Met (>99%; CJ BIO, Kerteh, Malaysia) at 0.05, 0.10, 0.15, or 0.2% of feed. During the grower phase, the BD contained 0.24% Met or was supplemented with DL-Met or L-Met at 0.04, 0.08, 0.12, or 0.16% of feed. The duck house was kept under a 24-h constant-light program. The temperature in the duck house was set at 33°C for the first 3 d, and then was reduced by 2°C each successive week until it settled at 24°C. Diets and water were supplied ad libitum in pellet form and by nipple drinkers, respectively. The dietary composition and nutrient levels of the BDs are listed in Table 1, with the Met levels of the 9 diets shown in Table 2. The crude protein and Met levels in corn, soybean meal, peanut meal and corn germ meal were measured before diet formulation. The Met levels in diet were analyzed accord-

Table 1. Dietary components and nutrient levels of the basal diet.

	$1 \ {\rm to} \ 14 \ {\rm d}$	$15\ {\rm to}\ 35\ {\rm d}$
Ingredients, % by weight		
Corn (CP, $7.78\%)^1$	61.14	67.67
Soybean meal (CP, 45.56%) ¹	26.00	10.50
Peanut meal (CP, 50.75%) ¹	5.00	12.00
Corn germ meal (CP, 18.56%) ¹	3.00	5.00
L-Lysine · HCl	0.28	0.36
L-Threonine	0.06	0.03
Soybean oil	0.60	0.60
Limestone	1.08	1.12
Dicalcium phosphate	1.60	1.42
NaCl	0.30	0.30
Premix ²	0.94	1.00
Total	100.00	100.00
Nutrient levels ³		
AME, Kcal/kg	2835	2900
Crude protein, %	19.70	17.80
Calcium, %	0.90	0.85
Non-phytate P, %	0.42	0.40
Lysine, %	1.20	1.00
Methionine, %	0.30	0.24
Methionine $+$ cysteine, $\%$	0.63	0.53
Threonine, %	0.76	0.58
Tryptophan, %	0.24	0.18

¹The crude protein levels are analyzed values.

²Provided per kg feed: VA 12,500 IU; VD₃ 4125 IU; VE 15 IU; VK 2 mg; thiamine 1 mg; riboflavin 8.5 mg; calcium pantothenate 50 mg; niacin 32.5 mg; pyridoxine 8 mg; biotin 2 mg; folic acid 5 mg; VB₁₂ 5 mg; Zn (ZnSO₄ · H₂O) 66 mg; I (KI) 1 mg; Fe (FeSO₄ · H₂O) 60 mg; Cu (CuSO₄ · 5H₂O) 8 mg; Se (Na₂SeO₃) 0.3 mg.

³Nutrient levels are calculated values.

 Table 2. Dietary Met supplemental levels and sources, and dietary Met contents.

	Met suppleme	ental levels, $\%$	Total dietary Met, $\%^1$				
Met source	1 to 14 d	$15\ {\rm to}\ 35\ {\rm d}$	1 to 14 d	$15\ {\rm to}\ 35\ {\rm d}$			
_	0	0	0.30(0.28)	0.24(0.25)			
DL-Met	$\begin{array}{c} 0.05 \\ 0.10 \\ 0.15 \\ 0.20 \end{array}$	$0.04 \\ 0.08 \\ 0.12 \\ 0.16$	$\begin{array}{c} 0.35 \ (0.34) \\ 0.40 \ (0.40) \\ 0.45 \ (0.44) \\ 0.50 \ (0.50) \end{array}$	$\begin{array}{c} 0.28 \ (0.29) \\ 0.32 \ (0.32) \\ 0.36 \ (0.36) \\ 0.40 \ (0.41) \end{array}$			
L-Met	$0.05 \\ 0.10 \\ 0.15 \\ 0.20$	$\begin{array}{c} 0.04 \\ 0.08 \\ 0.12 \\ 0.16 \end{array}$	$\begin{array}{c} 0.35 \ (0.33) \\ 0.40 \ (0.39) \\ 0.45 \ (0.45) \\ 0.50 \ (0.49) \end{array}$	$\begin{array}{c} 0.28 \; (0.28) \\ 0.32 \; (0.33) \\ 0.36 \; (0.37) \\ 0.40 \; (0.40) \end{array}$			

¹The number in parentheses is analyzed value.

ing to the Chinese National Standard method (GB/T 18,246–2000). Feed samples (1 g) were first hydrolyzed for 24 h in 6 mol/L hydrochloric acid (HCL, 10 mL) at 110°C. Hydrolysates were then diluted volumetrically to 50 mL with 0.02 mol/L HCL and 1 mL solution was dried in a water bath, then re-dissolved completely with 2 mL HCL (0.02 mol/L). Amino acids were analyzed in a fully-automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan) after passing through a 0.22 μ m filter.

Sample Collection

On days 14 and 35, 2 ducks around the average BW from each replicate were chosen and weighed and then were sacrificed by cervical dislocation, then part of the ileum was taken, rinsed in PBS and fixed in 10% buffered neutral formalin (Sinopharm Chemical Reagent Beijing Co., Ltd., Beijing, China) to assess morphological development.

On day 35, 2 ducks around the average BW from each replicate were chosen to measure feather traits. The ducks were then sacrificed by cervical dislocation and bled, de-feathered, and eviscerated to determine carcass weight (**CW**, without head, feet, or giblets). The abdominal fat and the breast and leg muscles of each duck were collected and weighed. Abdominal fat consisted of the adipose tissue from the proventriculus surrounding the gizzard down to the cloaca. Carcass weight yield was calculated as a percentage of total BW, whereas the breast and leg muscles yield were calculated as percentages of CW, and abdominal fat yield was calculated as a percentage of carcass plus abdominal fat weight.

Growth and Feather Traits

On days 1, 14, and 35, BW was recorded on a per replicate basis, and ADG was calculated for the starter and grower phases and the combined period. On days 14 and 35, feed consumption was determined as ADFI and F:G was calculated.

The length of the fourth primary wing feather was measured using a ruler with a minimum scale of 1 mm. Back feathers were scored subjectively using the criteria given in Table 3, according to the method described by Gustafson et al. (2007). The feather index was calculated as the ratio of weight of feathers relative to the weight of dressed carcass plus feathers.

Small Intestinal Morphology

The segments of the ileum were embedded in paraffin, transversely sectioned (5 μ m), and mounted on polylysine-coated slides. After de-waxing, sections 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) and crypt depth (from this junction to the base of the crypt) were determined (Shen

 Table 3. Feather score criteria.¹

Feature	Feather class	Score
No feathers on the back	0–	1
	0	2
	0+	3
Fewer feathers along the back	1-	4
0	1	5
	1 +	6
More feathers on the back	2-	7
	2	8
	2+	9
More and wider feathers on the back	3–	10
	3	11
	3+	12

¹According to the method described by Gustafson et al. (2007).

et al., 2009). Lengths of 10 well-oriented intact villi and their associated crypts were measured on each section.

Statistical Analysis

Each replicate pen was considered to be the experimental unit. The effect of dietary supplementation for each response variable was analyzed using the Mixed Model (PROC MIXED) procedure of SAS, version 8 (SAS Inst. Inc., Cary, NC). Preplanned contrasts were used to evaluate the effects of Met sources (BD vs. DL-Met, BD vs. L-Met, and L-Met vs. DL-Met using the average of 4 supplemental levels; Shen et al., 2015). Due to the nonlinear responses for growth and feather traits, nonlinear exponential regression analysis was used to evaluate the relative bioavailability (**RBV**) of L-Met to DL-Met (Littell et al., 1997; Shen et al., 2014; Xiao et al., 2015). The following nonlinear equation was applied: $y = a + b \times [1-Exp (c_1x_1 + c_2x_2)]$, in which y = variable (BW, ADG, F:G, etc.), a = intercept (value for the BD), b = asymptotic response, a + b = common asymptote (maximum level), $c_1 = slope$ ratio for DL-Met, c_2 = slope ratio for L-Met, and x_1 and x_2 = dietary supplemental level of DL-Met and L-Met, respectively. The RBV values of L-Met to DL-Met were given by the ratio of their c values $= c_2/c_1$. A 0.05 *P*-value was considered as being significant, unless otherwise stated.

RESULTS

Growth Performance

Compared with ducks fed the BD, both dietary DL-Met or L-Met supplementation increased BW of ducks at 14 and 35 d of age (P < 0.001, Table 4). During the starter phase (1 to 14 d), supplementation with either L-Met or DL-Met increased ADG and ADFI, and decreased F:G compared with ducks fed the BD (P <(0.05). In contrast, no difference in growth performance was observed in ducks supplemented with either DL-Met or L-Met for the grower phase (15 to 35 d; P >(0.05). For the entire 35 d period, supplementation with either L-Met or DL-Met had higher ADG than ducks fed the BD (P < 0.01). Ducks fed diets supplemented with L-Met tended to have decreased F:G than ducks fed the BD (P = 0.081), but there was no difference in F:G between the ducks fed the BD and DL-Met (P >(0.05). For the starter phase (days 1 to 14), there were increased efficacies of L-Met relative to DL-Met for BW (140.1%), ADG (137.6%) and feed efficiency (121.0%), reduced F:G) (Figure 1).

Carcass Yields

Ducks fed diets supplemented with L-Met had higher leg muscle proportion than that of ducks fed the BD (P < 0.01, Table 5) and it was also higher than the ducks fed diets supplemented with DL-Met (P < 0.05).

METHIONINE & PEKIN DUCK

Table 4. Growth performance of ducks fed graded levels of either DL-Met or L-Met compared to the basal diet (BD).¹

		Added	DL-Met, %	% (starter/	grower)	Added	L-Met, %	(starter/g		P-values			
Variable	BD	$\frac{0.05}{0.04}$	$\frac{0.10}{0.08}$	$\frac{0.15}{0.12}$	$\frac{0.20}{0.16}$	$\frac{0.05}{0.04}$	$\frac{0.10}{0.08}$	$\frac{0.15}{0.12}$	$\frac{0.20}{0.16}$	SEM	BD vs. DL-Met	BD vs. L-Met	L-Met vs. DL-Met
Initial BW, g 14 d BW, g 35 d BW, kg	54.0 490 2.28	$54.2 \\ 549 \\ 2.43$	$54.2 \\ 546 \\ 2.43$	$54.6 \\ 564 \\ 2.42$	53.7 578 2.44	54.2 553 2.42	$53.6 \\ 557 \\ 2.42$	$54.0 \\ 574 \\ 2.41$	54.2 580 2.47	$0.08 \\ 4.3 \\ 0.011$	0.389 < 0.001 < 0.001	0.934 < 0.001 < 0.001	0.220 0.269 0.938
Days 1 to 14 ADG, g ADFI, g F:G, g:g	$31.2 \\ 50.5 \\ 1.62$	$35.4 \\ 53.4 \\ 1.51$	$35.1 \\ 52.2 \\ 1.49$	$36.4 \\ 53.0 \\ 1.46$	$37.4 \\ 54.6 \\ 1.46$	$35.6 \\ 53.5 \\ 1.50$	$35.9 \\ 53.3 \\ 1.48$	$37.1 \\ 54.6 \\ 1.47$	$37.5 \\ 53.7 \\ 1.43$	$\begin{array}{c} 0.31 \\ 0.37 \\ 0.009 \end{array}$	$< 0.001 \\ 0.020 \\ < 0.001$	<0.001 0.008 <0.001	$0.253 \\ 0.588 \\ 0.587$
Days 15 to 35 ADG, g ADFI, g F:G, g:g	86.1 199 2.31	$89.9 \\ 203 \\ 2.27$	$89.9 \\ 205 \\ 2.28$	87.1 200 2.30	88.2 199 2.27	$87.3 \\ 204 \\ 2.34$	$89.7 \\ 199 \\ 2.22$	87.3 199 2.28	$90.8 \\ 206 \\ 2.27$	$0.56 \\ 1.0 \\ 0.017$	$0.167 \\ 0.381 \\ 0.614$	$\begin{array}{c} 0.163 \\ 0.367 \\ 0.611 \end{array}$	$\begin{array}{c} 0.981 \\ 0.966 \\ 0.996 \end{array}$
Days 1 to 35 ADG, g ADFI, g F:G, g:g	$62.6 \\ 135 \\ 2.16$	$66.5 \\ 139 \\ 2.10$	$66.2 \\ 139 \\ 2.10$	$65.5 \\ 138 \\ 2.10$	$66.6 \\ 138 \\ 2.07$	$65.2 \\ 140 \\ 2.15$	$66.5 \\ 136 \\ 2.05$	$65.7 \\ 137 \\ 2.08$	$67.8 \\ 140 \\ 2.07$	$\begin{array}{c} 0.35 \\ 0.5 \\ 0.013 \end{array}$	$0.002 \\ 0.105 \\ 0.102$	$\begin{array}{c} 0.001 \\ 0.124 \\ 0.081 \end{array}$	$0.909 \\ 0.892 \\ 0.855$

¹Each value is the mean of 6 replicates per treatment (20 ducks per replicate).



Figure 1. A, B, and C show BW at day 14, ADG, and F:G ratio between days 1 and 14 of ducks, D shows the back feathers score with increasing intake levels of either supplemental DL-Met (DLM) or L-Met (LM).

For breast muscle, the proportion was increased in ducks fed diets with DL-Met rather than L-Met supplementation (P < 0.001), and the former was higher than the latter (P < 0.01). There were no significant differences between the 2 sources of Met in CW percentage, dressing percentage, and abdominal fat (P > 0.05).

Feather Traits

Supplemental DL- or L-Met both increased the score of back feathers (P < 0.001, Table 5), and it tended to be higher in the ducks fed diets supplemented with L-Met rather than DL-Met (P = 0.081). Compared with the ducks given BD, the length of the fourth primary

ZHANG ET AL.

Table 5. Carcass qualities and feather traits of ducks fed graded levels of either DL-Met or L-Met compared to the basal diet (BD).¹

		Added I	DL-Met, %	6 (starter	/grower)	Added L-Met, $\%$ (starter/grower)					P-values		
Variable	BD	$\frac{0.05}{0.04}$	$\frac{0.10}{0.08}$	$\frac{0.15}{0.12}$	$\frac{0.20}{0.16}$	$\frac{0.05}{0.04}$	$\frac{0.10}{0.08}$	$\frac{0.15}{0.12}$	$\frac{0.20}{0.16}$	SEM	BD vs. DL-Met	BD vs. L-Met	L-Met vs. DL-Met
Carcass qualities													
Carcass percentage, $\%^2$	80.2	80.0	79.3	80.3	80.4	80.2	80.2	79.6	80.8	0.16	0.764	0.998	0.638
Dressing percentage, $\%^3$	77.4	78.0	77.3	77.1	77.6	76.5	77.7	77.2	76.9	0.16	0.881	0.513	0.207
Breast muscle yield, $\%^4$	10.5	11.7	12.0	11.1	11.5	10.7	11.2	11.3	10.7	0.10	< 0.001	0.088	0.003
Leg muscle yield, $\%^4$	15.2	15.6	15.7	15.1	15.5	15.5	15.6	15.9	16.6	0.09	0.220	0.007	0.015
Abdominal fat, $\%^4$	0.91	0.98	0.92	1.08	1.23	1.16	1.13	1.10	1.13	0.089	0.332	0.144	0.428
Feather traits													
Feather index, $\%^5$	8.35	8.59	9.34	8.46	8.45	8.35	8.35	9.02	8.99	0.181	0.575	0.608	0.939
Back feather score	4.50	4.92	5.29	5.54	5.67	5.21	5.42	5.83	5.83	0.079	< 0.001	< 0.001	0.094
Fourth primary length, cm	4.40	5.09	5.85	5.13	5.19	5.05	5.54	4.93	4.82	0.078	$<\!0.001$	0.002	0.090

¹Each value is the mean of 6 replicates per treatment (2 ducks per replicate).

²Percentage of BW at slaughter.

³Percentage of carcass weight after evisceration and without head, feet or giblets.

⁴Percentage of carcass plus abdominal fat weight.

⁵Percentage of the weight of dressed carcass plus feathers.

Table 6. Ileal mucosal morphology of ducks fed graded levels of either DL-Met or L-Met compared to the basal diet (BD).¹

	Added I	Added L-Met, % (starter/grower)					<i>P</i> -values						
Ileal mu cosal variable, $\mu {\rm m}$	BD	$\frac{0.05}{0.04}$	$\frac{0.10}{0.08}$	$\frac{0.15}{0.12}$	$\frac{0.20}{0.16}$	$\frac{0.05}{0.04}$	$\frac{0.10}{0.08}$	$\frac{0.15}{0.12}$	$\frac{0.20}{0.16}$	SEM	BD vs. DL-Met	BD vs. L-Met	L-Met vs. DL-Met
14 d Villus height 14 d Crypt depth 35 d Villus height 35 d Crypt depth	507 133 556 148	530 130 589 132	$535 \\ 125 \\ 643 \\ 136$	538 125 625 140	526 124 582 136	$550 \\ 135 \\ 605 \\ 140$	532 136 608 136	537 130 597 142	531 129 635 138	$4.6 \\ 1.8 \\ 6.0 \\ 1.5$	$\begin{array}{c} 0.003 \\ 0.237 \\ 0.004 \\ 0.020 \end{array}$	<0.001 0.900 0.003 0.073	0.329 0.097 0.879 0.363

¹Each value is the mean of 6 replicates per treatment (2 ducks per replicate).

wing feather was increased with DL-Met and L-Met supplementation (P < 0.01), and there was a tendency for it to be increased with DL-Met over that with L-Met (P = 0.090). For the feather traits, there was an increased efficacy of L-Met relative to DL-Met for back feathers score (153.1%), but no appropriate regression could be used in the length of the fourth primary feather (Figure 1).

Small Intestinal Morphology

At d 14, ducks supplemented with either DL-Met or L-Met had greater villus height in the ileum than ducks fed BD (P < 0.01, Table 6). Dietary DL-Met tended to decrease ileal crypt depth compared with L-Met (P =0.097). At day 35, dietary DL-Met or L-Met resulted in increased ileal villus height compared with BD (P <0.01). Supplementation of ducks with DL-Met reduced (P < 0.05), while L-Met tended to reduce (P = 0.073) ileal crypt depth compared to the ducks fed with the BD.

DISCUSSION

As expected, the present study with young meattype ducks found that either DL- or L-Met added to the diet improved their growth performance, presumably because of its positive effects on protein synthesis, methyl donation, and antioxidant activity (Luo and Levine, 2009). The beneficial effects of dietary supplementation with either source of Met on growth performance were realized in the starter rather than grower phase. It probably reflects a different Met requirement of ducks in the starter and grower phases, as it has been reported that to obtain the maximum weight gain of Pekin ducklings, same breed as used here, dietary Met need to be 0.481% from hatch to 21 d of age (Xie et al., 2004), higher than the 0.377% needed between 21 and 49 d of age (Xie et al., 2006). In addition, there was no obvious difference in growth performance between supplementation with DL- or L-Met in meat ducks, just as found in broiler chickens from 8 to 20 d of age (Dilger and Baker, 2007). Excessive supplementation with either DL- or L-Met was equally detrimental for starter Pekin ducks (Xue et al., 2018). In the present study, dietary supplementation for the whole period with L-Met rather than DL-Met tended to increase feed efficiency of ducks. Noteworthy was that the RBV of L-Met to DL-Met ranged from 120 to 140% for growth performance during the starter phase (Figure 1). These results imply that L-Met had better bioavailability than DL-Met, perhaps because L-Met can be used directly

in protein synthesis whereas part of DL-Met requires deamination to keto-methionine and then transamination to L-Met before it can be incorporated into protein (Dilger et al., 2007). Linda (1985, 1987) reported that DL-Met and L-Met were metabolized differently in broiler chicks and their abilities to act as precursors for protein synthesis differed. Consistent with that, Shen et al. (2015) found that supplementation with L-Met gave a better growth response than DL-Met in chicks (0 to 21 d), mainly because of improved redox status and development of the gut; the same was found in turkey poults (Park et al., 2018). The present findings indicated that dietary supplementation of ducks with DL-Met or L-Met improved growth performance, especially in the starter phase, and the efficacy of L-Met relative to DL-Met ranged from 120 to 140.0%.

Consistent with several earlier studies, dietary supplementation here with DL-Met for 35 d increased the breast muscle yield of ducks. Jamroz et al. (2009) found that supplementation of a diet containing 0.28% Met with 0.12% DL-Met had a positive effect on the productive output of meat ducks from 1 to 42 d age, and ducks fed 0.47% (1 to 21 d) and 0.42% (22 to 41 d) Met had markedly higher breast meat yield at 41 d (Wang et al., 2004). In addition, the breast meat yield of ducks showed a quadratic response to DL-Met supplementation; for example, increasing total dietary Met between 0.20 and 0.575% (21 to 49 d, Xie et al., 2006) or between 0.30 and 0.68% (15 to 35 d, Zeng et al., 2015). A novel finding from the present study was that leg muscle vield of ducks was increased by feeding additional L-Met for 35 d. Few studies have investigated the effect of L-Met or its comparison to DL-Met on carcass vields in ducks. The different effects of DL-Met and L-Met observed here on yields of these 2 major products (breast and leg muscle) indicates different functions in protein synthesis and metabolism of the 2 forms of Met, as it known that breast and leg muscle are different types and develop differently during post-hatch stages in Pekin ducks (Xu et al., 2013). Leg and breast muscle consist of different fiber types, which varied in many aspects such as protein constitution, physical properties, glycolysis metabolism, and taste, all of which are related to meat production (Zhang et al., 2009; Xu et al., 2013; Wang et al., 2017). In this regard, the different roles of DL-Met and L-Met in protein synthesis and to distinct muscle fibers might account for the different effects on breast and leg muscle vield demonstrated here. It was not possible in the present study to compare the relative efficacy of L-Met and DL-Met on yields of breast and leg muscle because there was no regression of these traits on increasing supplemental levels of Met.

In the current study, dietary supplementation with DL-Met or L-Met increased the back feathers score and the length of the fourth wing feather compared with the BD, indicating that insufficient Met impaired feather growth of these meat ducks. It has been reported that

dietary supplementation of meat ducks with DL-Met increased total and relative feather weight and coverage (Guo, 2011; Zeng et al., 2015). Met is required for synthesis of keratin, the major component of feathers of waterfowl and predominantly composed of sulfur amino acid (SAA, 12%), the most important amino acids for its synthesis (Wheeler and Latshaw, 1981); the SAA content of dry ground contour feathers of ducks was 5.1% (Zheng and Zhang, 1989). Dietary supplementation with SAA improved the yield and sulfur content of waterfowl feathers (Wheeler and Latshaw, 1981). In the present study, back feathers and fourth wing feather length were increased with both L-Met and DL-Met; L-Met appeared to be better for back feather growth while DL-Met tended to result in longer fourth wing feathers. As for previously noted differences in other traits, this finding suggests that DL-Met and L-Met might have differential effects in feather growth, and the efficacy of L-Met to DL-Met was 153% for the back feathers at d 35.

Methionine is a specific functional essential AA in the growth and development of the gastrointestinal tract of animals (Stoll et al., 1998), and its functional role, especially its antioxidative effect, may be critical for the health of the gastrointestinal tract of a rapidly growing animal and thus its growth (Shen et al., 2014). In the current study, ducks fed diets supplemented with DL-Met or L-Met had better villus morphology in the ileum than the ducks fed the BD, mainly reflecting improved development, as shown by increased villus height and decreased crypt depth. This result agrees with Shen et al. (2015), who reported the supplementation of young chickens with either L-Met or DL-Met improved villus development and redox status of the duodenum. In this respect, the improved ileal mucosal development demonstrated here in ducks possibly resulted from the antioxidative role of Met, as it was reported that supplemental Met in nursery pig diets enhanced duodenal villus development in association with reduced oxidative stress and improved GSH (Shen et al., 2014). The studies with piglets found L-Met to be superior to DL-Met in improving gut development (Shen et al., 2014; Shen et al., 2015); this difference was in accordance with better growth performance of L-Met compared with DL-Met in chicks or nursery pigs. The current study using ducks showed small intestinal morphology was improved with Met supplementation and there were no significant differences in between DL-Met and L-Met, which was consistent with growth performance being enhanced by supplementation with Met, but without differences between the 2 forms used.

In conclusion, dietary supplementation with L-Met or DL-Met increased the growth performance of meat ducks, especially during the starter phase, and improved the feather traits and small intestinal morphology. The efficacy of supplemental L-Met to DL-Met ranged from 120 to 140% for growth performance traits of young ducks (1 to 14 d) and was 153% for the feather traits of ducks (35 d).

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REFERENCES

- Chung, T. K., and D. H. Baker. 1992. Utilization of methionine isomers and analogs by the pig. Can. J. Anim. Sci. 72:185–188.
- Dilger, R. N., and D. H. Baker. 2007. DL-Methionine is as efficacious as L-methionine, but modest L-cystine excesses are anorexigenic in sulfur amino acid-deficient purified and practical-type diets fed to chicks. Poult. Sci. 86:2367–2374.
- Dilger, R. N., C. Kobler, C. Weckbecker, D. Hoehler, and D. H. Baker. 2007. 2-Keto-4-(methylthio) butyric acid (keto analog of methionine) is a safe and efficacious precursor of DL-methionine in chicks. J. Nutr. 137:1868–1873.
- Georgiev, T., A. Ratkov, J. Kristeva, V. Ivanova, A. Dimitrova, A. Manchev, and B. Ratkov. 2002. Improved mathematical model of L-lysine fermentation process based on manure. Proc. 24th Int. Conf. Infor. Tech. Interfaces. 401.
- Guo, F. 2011. Effects of methionine on feather development of started Peking ducks of 0 to 21d of age. MS Thesis. The Chinese Academy of Agricultural Sciences, Beijing, China.
- Gustafson, L. A., H. W. Cheng, J. P. Garner, E. A. Pajor, and J. A. Mench. 2007. The effects of different bill-trimming methods on the well-being of Pekin ducks. Poult. Sci. 86:1831–1839.
- Jamroz, D., A. Wiliczkiewicz, A. Lemme, J. Orda, J. Skorupińska, and T. Wertelecki. 2009. Effect of increased methionine level on performance and apparent ileal digestibility of amino acids in ducks. J. Anim. Physiol. An. N. 93:622–630.
- Kluge, H., D. K. Gessner, E. Herzog, and K. Eder. 2016. Efficacy of DL-methionine hydroxy analogue-free acid in comparison to DL-methionine in growing male white Pekin ducks. Poult. Sci. 95:590–594.
- Kong, C., C. S. Park, J. Y. Ahn, and B. G. Kim. 2016. Relative bioavailability of DL-methionine compared with L-methionine fed to nursery pigs. Anim. Feed Sci. Tech. 215:181–185.
- Lemme, A., D. Hoehler, J. Brennan, and P. Mannion. 2002. Relative effectiveness of methionine hydroxy analog compared to DLmethionine in broiler chickens. Poult. Sci. 81:838–845.
- Linda, S. C. 1985. Comparative metabolism of L-methionine, DL-methionine and DL-2-hydroxy 4-methylthiobutanoic acid by broiler chicks. Brit. J. Nutr. 54:621–633.
- Linda, S. C. 1987. Effect of fasting and of methionine deficiency on L-methionine, DL-methionine and DL-2-hydroxy-4methylthiobutanoic acid metabolism in broiler chicks. Brit. J. Nutr. 57:429–437.
- Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. J. Anim. Sci. 75:2672–2683.
- Lopez-Coello, C. 2003. Potential causes of broiler feathering problems. Feathering Manual. Novus International, St. Louis, MO. P. 1–46.
- Luo, S., and R. Levine. 2009. Methionine in proteins defends against oxidative stress. FASEB J. 23:464–472.
- Mandal, A. B., A. V. Elangovan, and T. S. Johri. 2004. Comparing bio-efficacy of liquid DL-methionine hydroxy analogue free acid with DL-methionine in broiler chickens. Asian Austral. J. of Anim. Sci. 17:102–108.
- Park, I., T. Pasquetti, R. D. Malheiros, P. R. Ferket, and S. W. Kim. 2018. Effects of supplemental L-methionine on growth performance and redox status of turkey poults compared with the use of DL-methionine. Poult. Sci. 97:102–109.

- Powell, C. D., M. A. K. Chowdhury, and D. P. Bureau. 2017. Assessing the bioavailability of L-methionine and a methionine hydroxy analogue (MHA-Ca) compared to DL-methionine in rainbow trout (*Oncorhynchus mykiss*). Aquac. Res. 48:332–346.
- Shen, Y. B., A. C. Weaver, and S. W. Kim. 2014. Effect of feed grade L-methionine on growth performance and gut health in nursery pigs compared with conventional DL-methionine. J. Anim. Sci. 92:5530–5539.
- Shen, Y. B., P. Ferket, I. Park, R. D. Malheiros, and S. W. Kim. 2015. Effects of feed grade L-methionine on intestinal redox status, intestinal development, and growth performance of young chickens compared with conventional DL-methionine. J. Anim. Sci. 93:2977–2986.
- Shen, Y. B., X. S. Piao, S. W. Kim, L. Wang, P. Liu, I. Yoon, and Y. G. Zhen. 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J. Anim. Sci. 87:2614–2624.
- Stoll, B., J. Henry, P. J. Reeds, H. Yu, F. Jahoor, and D. G. Burrin. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J. Nutr. 128:606–614.
- Wang, X. F., J. L. Li, J. H. Cong, X. X. Chen, X. D. Zhu, L. Zhang, F. Gao, and G. H. Zhou. 2017. Preslaughter transport effect on broiler meat quality and post-mortem glycolysis metabolism of muscles with different fiber types. J. Agr. Food Chem. 65:10310– 10316.
- Wang, Y. Z., Z. R. Xu, and J. Feng. 2004. The effect of betaine and DL-methionine on growth performance and carcass characteristics in meat ducks. Anim. Feed Sci. Technol. 116:151– 159.
- Wheeler, K. B., and J. D. Latshaw. 1981. Sulfur amino acid requirements and interactions in broilers during two growth periods. Poult. Sci. 60:228–236.
- Xiao, J. F., S. G. Wu, H. J. Zhang, H. Y. Yue, J. Wang, F. Ji, and G. H. Qi. 2015. Bioefficacy comparison of organic manganese with inorganic manganese for eggshell quality in Hy-Line Brown laying hens. Poult. Sci. 94:1871–1878.
- Xie, M., S. S. Hou, and W. Huang. 2006. Methionine requirements of male white Peking ducks from twenty-one to forty-nine days of age. Poult. Sci. 85:743–746.
- Xie, M., S. S. Hou, W. Huang, and H. P. Fan. 2007. Effect of excess methionine and methionine hydroxy analogue on growth performance and plasma homocysteine of growing pekin ducks. Poult. Sci. 86:1995–1999.
- Xie, M., S. S. Hou, W. Huang, L. Zhao, J. Y. Yu, W. Y. Li, and Y. Y. Wu. 2004. Interrelationship between methionine and cystine of early Peking ducklings. Poult. Sci. 83:1703–1708.
- Xu, T. S., L. H. Gu, X. H. Zhang, W. Huang, B. G. Ye, X. L. Liu, and S. S. Hou. 2013. IGF-1 and FoxO3 expression profiles and developmental differences of breast and leg muscle in pekin ducks (*Anas platyrhynchos domestica*) during postnatal stages. J. Anim. Vet. Adv. 12:852–858.
- Xue, J. J., M. Xie, J. Tang, W. Huang, Q. Zhang, and S. S. Hou. 2018. Effects of excess DL-and L-methionine on growth performance of starter Pekin ducks. Poult. Sci. 97:946– 950.
- Yi, G. F., A. M. Gaines, B. W. Ratliff, P. Srichana, G. L. Allee, K. R. Perryman, and C. D. Knight. 2006. Estimation of the true ileal digestible lysine and sulfur amino acid requirement and comparison of the bioefficacy of 2-hydroxy-4-(methylthio)butanoic acid and DL-methionine in eleven- to twenty-six-kilogram nursery pigs. J. Anim. Sci. 84:1709–1721.
- Zeng, Q. F., Q. Zhang, X. Chen, A. Doster, R. Murdoch, M. Makagon, A. Gardner, and T. J. Applegate. 2015. Effect of dietary methionine content on growth performance, carcass traits, and feather growth of Pekin duck from 15 to 35 days of age. Poult. Sci. 94:1592–1599.
- Zhang, L., H. Y. Yue, H. J. Zhang, L. Xu, S. G. Wu, H. J. Yan, Y. S. Gong, and G. H. Qi. 2009. Transport stress in broilers: I. Blood metabolism, glycolytic potential, and meat quality. Poult. Sci. 88:2033–2041.
- Zheng, W. Z., and S. Z. Zhang. 1989. Comparative analysis of the composition of duck's feather. Nat. Sci. Xiamen University, China. 28:667–671. (in Chinese).