Effects of L-Met and DL-Met on growth performance of medium-growing yellow -feathered chickens between 1-30 days of age

Abstract

This experiment investigated the effects of L-Methionine (L-Met) on growth performance and feather traits, and compared with DL-Met in medium-growing yellow-feathered broilers during 1 to 30 days of age. A total of 1,584 1-day old broilers were randomly divided into 11 treatment groups with 6 replicates of 24 birds each: basal diet (CON, Met 0.28%), basal diet + L-Met (0.04%, 0.08%, 0.12%, 0.16%, 0.20%), basal diet + DL-Met (0.04%, 0.08%, 0.12%, 0.16%, 0.20%). Compared with broilers fed the basal diet, dietary with 0.04 to 0.20% supplemental Met increased the final body weight (FBW), average daily feed intake (ADFI), average daily gain (ADG) and decreasing feed to gain ration (F/G) ($P < 0.05$).
According to the ADG, ADFI and F/G of 1-30 d, the relative biological value (RBV) of L-Met compared with DL-Met were 172.4%, 88.2% and 283.0%, respectively. Compared with the control group, supplementation with 0.20% L-Met and not with DL-Met significantly increased the length of the fourth primary wing feather and score of moulting ($P < 0.05$). In conclusion, Dietary supplementation with L-Met or DL-Met improved the growth performance, and feather traits of yellow-feathered broilers aged 1 to 30 d, was improved only when L-Met was supplemented. The relative bioavailability (RBV) of L-Met was higher than DL-Met. From the quadratic regressions, the optimal supplementation L-Met and DL-Met of medium-growing yellow-feathered broilers at the started phase to achieve the best performance (ADG, F/G) were 0.44% Met (supplementation 0.16% L-Met) and 0.48% Met (supplementation 0.20% DL-Met), the daily Met requirement was 0.152g and 0.168g based on L-Met and DL-Met, and the ratio of Lys to Met were 100:42 and 100:46, respectively.

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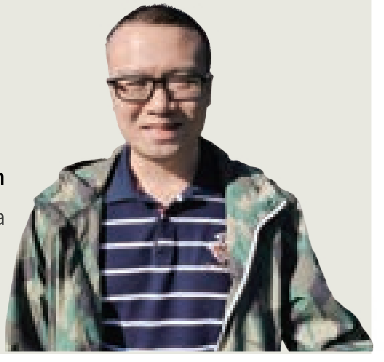

Background

Methionine (Met), the first limiting amino acid in broiler diet, plays an important role in protein synthesis (Lemme et al., 2020), as a methyl donor (Parkhitko et al., 2019), and in cell proliferation (Tsiagbe et al., 1987). Diets with inadequate Met content can have a negative impact on growth performance (Seifalinasab et al., 2022), carcass quality (Majdeddin et al., 2019), antioxidant capacity (Martinez et al., 2017) and lipid metabolism (Moghadam et al., 2017). Therefore, the precise requirement of Met has particularly importance for broiler production (Xue et al., 2018; Fagundes et al., 2020).

At present, DL-methionine (DL-Met) is the most commonly used sources of Met, which is a 50:50 percent composition of D-Met and L-Met produced via chemical synthesis. L-methionine (L-Met) is the natural form of Met produced via fermentation. Thus, it can be directly absorbed and utilized by animals (Georgiev et al., 2002). However, D-Met cannot be used directly which means it first needs to be converted to L-Met by D-Amino acid oxidase (D-AAO) (Brachet et al., 1992). The two isomers of Met (D- and L-isomer) compete sometimes for the same transporter, but L-Met has a much higher affinity for most of the transporters (Yi et al., 2006). The absorption rate of D-Met and DL-Met in the gut is significantly slower than that of L-Met, and the difference in absorption rate between D-Met and L-Met was particularly obvious under heat stress (Richards et al., 2005).

The efficacy of L-Met in comparison to DL-Met has been examined recently in fast-growing chickens (Shen et al., 2015) or other species (Powell et al., 2017; Park et al., 2018; Zhang et al., 2019). These studies reported the advantages of L-Met compared with DL-Met. Yellow-feathered chickens are very important in China, which 4 billion marketsize annually (Tang et al., 2021). L-Met requirements of yellow-feathered broilers remains not fully elucidated at different growth rate. The aim of this trial was to look at the effects of different levels and sources of Met on growth performance and feather traits of medium-growing yellow-feathered chickens during the starter phase and further provides a rational recommendation for the appropriate dietary Met levels for yellow-feathered chickens.

Effects of L-Met and DL-Met on growth performance of medium-growing yellow -feathered chickens between 1-30 days of age

Materials and methods

Birds, Experimental Design and Diets

The experimental protocol was approved by the Animal Care Committee of the Institute of Animal Science, Guangdong Academy of Agriculture Science, Guangzhou, P. R. China, with the approval number of GAASISA-2015-020.

A total of 1584 1 day (d), yellow-feathered chickens (medium-growing strain) were randomly divided into 11 treatment groups (Table 1), each with 6 replicates of 24 broilers. Birds in the control group (CON) were fed a basal diet (CON, Met 0.28%), and birds in the other 10 treatments received the basal diet with added L-Met (0.04%, 0.08%, 0.12%, 0.16%, 0.20%) or DL-Met (0.04%, 0.08%, 0.12%, 0.16%, 0.20%). Birds were raised in floor pens with wood shavings litter, with the stocking density of 0.20 m2/bird. The room temperature was kept at 32 to 34°C for the first 3 days and then reduced by 2°C per week until settled at 28°C. Diets and water were supplied ad libitum throughout the experiment. The diets were formulated according to Chinese Nutrient Requirements of Yellow Chickens (Ministry of Agriculture and Rural Affairs, 2020), with the exception of Met. Details of ingredient composition and nutrient contents of the experimental diets for chickens are provided in Table 2.

Table 1. Experimental design

Treatment	Met source	Met supplemental level (%)	Total dietary Met (%)
T0 (Control)	Basal diet	0.00	0.28
T1	DL-Met	0.04	0.32
T2		0.08	0.36
T3		0.12	0.40
T4		0.16	0.44
T5		0.20	0.48
T6	L-Met	0.04	0.32
T7		0.08	0.36
T8		0.12	0.40
T9		0.16	0.44
T10		0.20	0.48

Table 2. Composition and nutrient levels of the basal diet

Ingredients	%	Nutrient levels[2]	%
Corn	60.39	AME(MJ/kg)	11.92
Soybean meal	25.05	CP	19.90
Peanut meal	5.00	Ca	1.00
Pea protein powder	2.41	P	0.74
Soybean oil	2.74	Lys	1.05
$CaHPO_4$	1.68	Met	0.28
NaCl	0.30	Met+Cys	0.59
Limestone	1.09	Thr	0.72
L-Lys·HCl	0.10	Trp	0.22
L-Met	0.00	Arg	1.40
DL-Met	0.00	Ile	0.77
Rice bran	0.24	Val	0.90
Premix[1]	1.00		
Total	100.00		

[1]Premix provided the following per kilogram of the diet: VA 12 000 IU, VD_3 600 IU, VE 45 IU, VK_3 2.5 mg, VB_1 1.8 mg, VB_2 9.0 mg, VB_6 2.8 mg, VB_{12} 16 mg, choline 1300 mg, niacin 42 mg, pantothenic acid 16 mg, folic acid 1.0 mg, biotin 0.12 mg, Fe 80 mg, Cu 18.8 mg, Mn 60 mg, Zn 80 mg, I 0.7 mg, Se 0.15 mg.

[2]AME was calculated values which based on the data presented in the Chinese feed database (Chinese feed database. 2021), while the others were measured values.

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Materials and methods

Measurement of Growth Performance

Initial and final body weights were recorded per replicate on d 1 and 30 of age, respectively. Mortality was checked daily, and dead birds were recorded and weighed to adjust estimates of gain and intake. Average daily body weight gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F/G) was calculated on a replicate basis from 1 to 30 d.

Table 3. Scoring criteria of feather traits[1]

Molting degree	Feather class	Score
The back, breast and abdomen have not yet moulted	0-	1
	0	2
	0+	3
A few on the back and breast and abdomen began to molt	1-	4
	1	5
	1+	6
The back and breast and abdomen are moulting, and there are more down feathers left	2-	7
	2	8
	2+	9
The back, breast and abdomen are more moulting, and there are less down feathers left	3-	10
	3	11
	3+	12

[1]According to the Davis et al. (2004)

Evaluation of Feather Traits

At 29 d, 30 chickens (5 birds per replicate) were randomly chosen from each treatment group for feather status measurement. The length of the fourth primary wing feather was measured using a ruler with a minimum scale of 1 mm. The feathers of abdomen, back and breast were scored subjectively using the criteria given in Table 3, according to Davis et al. (2004).

Statistical Analysis

Replicates or individual birds were taken as the experimental unit. The effects of dietary Met treatment were assessed by one-way ANOVA procedures of SAS (version 8.0). Preplanned contrasts were used to evaluate the effects of Met sources (CON vs. DL-Met, CON vs. L-Met, and DL-Met vs. L-Met). When treatment effects were significant ($P < 0.05$), means were separated by Duncan's multiple range test. Tabulated results were shown as means with standard error of mean (SEM). For key performance variables (ADFI, ADG, F/G), the dietary Met requirement of the birds was estimated using quadratic polynomial (QP) models by the NLIN procedure of SAS. QP model: $Y=c+bX+aX^2$, where a = quadratic coefficient, b = linear coefficient, c = intercept. The requirement of Met was defined as Met = $-b/(2 \times a)$. Nonlinear exponential regression analysis was used to evaluate the relative bioavailability (RBV) of L-Met and DL-Met (Littell et al., 1997; Shen et al., 2014). $Y=a + b \times (1–EXP–(c_1X_1 + c_2X_2))$, in which y = variable (ADFI, ADG, F/G), a = intercept (value for the CON), b = asymptotic response, a + b = common asymptote (maximum growth performance level), c_1 = slope ratio for DL-Met, c_2 = slope ratio for L-Met, and X1 and X_2 = dietary supplemental level of DL-Met and L-Met, respectively.
The RBV of L-Met and DL-Met were given by the ratio of their c values = $[100 \times (c_2/c_1)]$ according to Littell et al. (1997).

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Results

Effects of Dietary Met sources and supplemental levels on growth performance

Compared with the control group, 0.04 to 0.20% Met addition increased FBW, ADFI, ADG and improved F/G ($P < 0.05$). There were interactions ($P < 0.05$) between levels and sources of Met on the FBW, ADFI and ADG.

Table 4. Effects of Met source and supplemental levels on growth performance of yellow-feathered broilers from 1 to 30 days of age[1]

Item: Met source	Item: Supplemental Dietary Met level	IBW (g)	30d FBW (g)	ADFI (g/d)	ADG (g/d)	F/G	Mortality (%)
DL-Met	0.00	38.79	486.08^{f}	31.81^{e}	15.97^{e}	2.00^{a}	1.39
	0.04	38.81	563.85^{e}	34.75^{bcd}	18.75^{e}	1.87^{b}	1.39
	0.08	38.81	570.09^{e}	34.4^{cd}	18.97^{e}	1.82^{bc}	1.39
	0.12	38.78	616.85^{ab}	36.99^{a}	20.73^{a}	1.82^{bc}	3.47
	0.16	38.83	579.86^{de}	33.87^{a}	19.3^{cd}	1.76^{cd}	1.39
	0.20	38.82	613.20^{abc}	34.99^{bcd}	20.51^{ab}	1.71^{d}	0.00
L-Met	0.04	38.82	587.50^{cde}	35.06^{bcd}	19.57^{bcd}	1.79^{bcd}	0.00
	0.08	38.79	587.42^{cde}	33.92^{d}	19.61^{bcd}	1.74^{cd}	0.69
	0.12	38.80	597.45^{bcd}	34.60^{bcd}	19.95^{abc}	1.74^{cd}	1.39
	0.16	38.85	626.03^{a}	36.41^{ab}	20.97^{a}	1.77^{cd}	2.78
	0.20	38.84	618.53^{ab}	36.03^{abc}	20.70^{a}	1.76^{cd}	2.08
SEM		0.032	9.069	0.610	0.324	0.029	0.836
Main Effect							
Met source	DL-Met	38.81	589.23^{b}	35.00	19.66^{b}	1.80	1.53
	L-Met	38.82	603.31^{a}	35.20	20.16^{a}	1.76	1.39
Met level (%)	0.00	38.79	486.08^{d}	31.81^{c}	15.97^{d}	2.00^{a}	1.39
	0.04	38.81	575.33^{bc}	34.90^{ab}	19.16^{bc}	1.83^{b}	0.69
	0.08	38.80	578.93^{b}	34.16^{b}	19.29^{b}	1.78^{bc}	1.04
	0.12	38.79	608.29^{a}	35.80^{a}	20.34^{a}	1.78^{bc}	2.43
	0.16	38.84	602.94^{a}	35.14^{a}	20.15^{a}	1.77^{c}	2.08
	0.20	38.83	615.86^{a}	35.51^{a}	20.61^{a}	1.73^{c}	1.04
Sources of variation							
Supplemental Met level		0.433	<0.001	<0.001	<0.001	<0.001	0.277
Met Source		0.694	0.029	0.631	0.029	0.082	0.811
Level×source		0.982	0.012	0.007	0.012	0.100	0.117

$^{a\text{-}f}$Within a column, means with different superscripts differ significantly ($P < 0.05$).

[1]Data are means of 6 replicates with 24 birds per replicate for the interaction.
For the main effects of Met source, data are means of 30 replicates with 24 birds per replicate; the main effects of Met supplemental level, data are means of 12 replicates with 24 birds per replicate.

There were increased efficacies of L-Met relative to DL-Met for ADG, ADFI and F/G, 172.4%, 88.2% and 283.0%, respectively (Table 5).

Table 5. The RBV of L-Met in comparison to DL-Met of ADG of yellow-feathered broilers from 1 to 30 days of age

Evaluation indicators	Regression equation[1]	R^2	RBV
ADG	$Y=16.025+4.374\times[1-Exp-(19.838X_1+34.202X_2)]$	0.913	172.4%
ADFI	$Y=31.850+3.436\times[1-Exp-(40.649X_1+35.853X_2)]$	0.660	88.2%
F/G	$Y=1.9968-0.2528\times[1-Exp-(15.4828X_1+43.8156X_2)]$	0.955	283.0%

ADG = average daily gain; ADFI = average daily feed intake; F/G = Feed to gain ratio; RBV= relative bioavailability.

[1]$Y= a + b \times (1 - EXP - (c_1X_1 + c_2X_2))$, in which y = variable (ADFI, ADG, F/G), a = intercept (value for the CON), b = asymptotic response, a + b = common asymptote (maximum growth performance 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 of L-Met and DL-Met were given by the ratio of their c values = $[100 \times (c_2 / c_1)]$.

According to the growth performance (ADF and F/G), the optimal L-Met level and DL-Met level from the quadratic regressions (quadratically, $P < 0.05$) were 0.44% and 0.48% (Table 6).

Table 6. Optimum methionine supplementation of yellow-feathered broilers from 1 to 30 days of age

Evaluation indicators	Met source	Regression equation[1]	R^2	P value	The optimal Met supplemental group (%)	Met recommended (%)	Daily requirements (g)
ADG	DL-Met	$Y=-165.91X^2+52.76X+16.27$	0.76	<0.001	0.20	0.48	0.165
	L-Met	$Y=-167.25X^2+54.46X+16.40$	0.76	<0.001	0.16	0.44	0.152
F/G	DL-Met	$Y=7.54X^2-2.91X+1.99$	0.87	<0.001	0.20	0.48	0.165
	L-Met	$Y=14.68X^2-3.85X+1.97$	0.77	<0.001	0.16	0.44	0.152

[1]$Y=c + bX + aX^2$, where a = quadratic coefficient, b = linear coefficient, c = intercept.
The requirement of Met was defined as Met = $-b / (2 \times a)$.

Effects of L-Met and DL-Met on growth performance of medium-growing yellow -feathered chickens between 1-30 days of age

Results

Effects of dietary Met sources and supplemental levels on feather traits

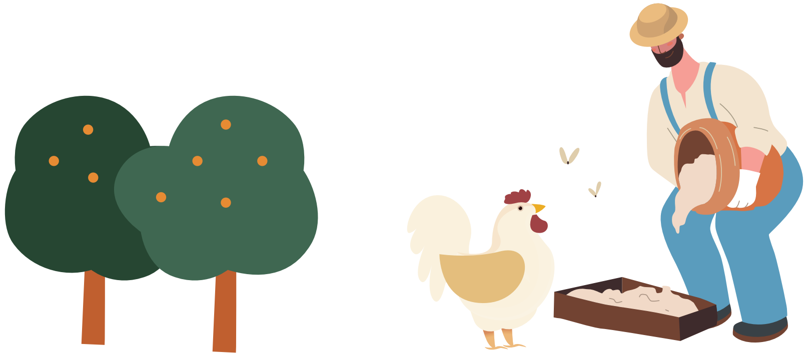
The effects of different sources and levels of Met on feather traits are presented in Table 7. Dietary supplementaion with 0.20% L-Met increased the length of the fourth primary wing feather and the score of moulting compared with the control group ($P < 0.05$).

Compared with DL-Met group, L-Met group increased the length of the fourth primary feather and the score of moulting ($P < 0.05$), where the highest values were observed with 0.20% supplemental L-Met in the starter phase. Significant inter-actions between levels and sources existed for the length of the fourth wing feather and score of moulting ($P < 0.05$).

Table 7. Effects of Met source and supplemental levels on feather development of yellow-feathered broilers at 30 days of ages[1]

Item	Met Source						Met Source					SEM	Main effects								Sources of variation		
	DL-Met						L-Met						Met Source DL-Met	Met Source L-Met	Met supplemental level						Met Source	Supplemental Met level	Level × source
Met supplemental level	0.00	0.04	0.08	0.12	0.16	0.20	0.04	0.08	0.12	0.16	0.20				0.00	0.04	0.08	0.12	0.16	0.20			
Length of the fourth primary feather (cm)	6.78^{b}	6.71^{b}	6.55^{b}	6.71^{b}	6.79^{b}	6.79^{b}	6.80^{b}	7.79^{a}	6.77^{b}	6.97^{b}	8.14^{a}	0.222	6.72^{b}	7.21^{a}	6.78^{b}	6.76^{b}	7.17^{ab}	6.74^{b}	6.88^{b}	7.46^{a}	0.0004	0.0085	0.0035
Score of moulting	7.39^{cd}	7.48^{cd}	7.19^{d}	7.32^{cd}	7.25^{d}	7.45^{cd}	7.63^{c}	8.20^{b}	7.37^{cd}	7.43^{cd}	10.87^{a}	0.113	7.35^{b}	8.15^{a}	7.39^{b}	7.56^{b}	7.70^{b}	7.35^{b}	7.34^{b}	9.16^{a}	<.0001	<.0001	<.0001

[a-d] Within a row, means with different superscripts differ significantly ($P < 0.05$).
[1]Data are means of 6 replicates with 5 birds per replicate for the interaction.
For the main effects of Met source, data are means of 30 replicates with 5 birds per replicate;
the main effects of Met supplemental level, data are means of 12 replicates with 5 birds per replicate.



Effects of L-Met and DL-Met on growth performance of medium-growing yellow -feathered chickens between 1-30 days of age

Discussion

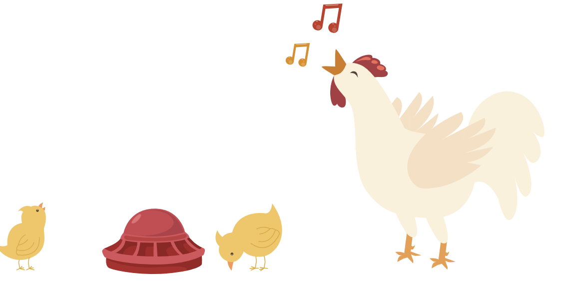
Methionine is an essential amino acid for animals (especially poultry) and an important substance for protein synthesis (Luo and Levine, 2009). Increasing dietary Met levels can significantly improve ADG and F/G of broilers (Dilger and Baker, 2007; Li et al., 2021; Zeitz et al., 2019). In the current study, dietary Met improved the growth performance of medium-growing yellow-feathered broilers during the starter phase, and the growth performance of L-Met supplementation groups was significantly higher than that of DL-Met supplementation groups. It suggested a difference of efficacy between L-Met and D-Met. L-methionine can be directly absorbed and utilized by the body, while D-Met needs D-AAO for conversion which has a different activity depending of species, tissue and age (Zhang et al., 2018). The current study showed that the efficacy of L-Met was higher than DL-Met. Some previous studies had similar findings, Shen et al. (2015) showed that the RBV of L-Met compared to D-Met was 140.7% in broilers. Zhang et al. (2019) found that the RBV of L-Met in Cherry Valley ducks compared to DL-Met was 120-140% based on ADG and F/G.

Feathers are mostly made of keratin, and the properties of keratin depend on the amount of sulfur-containing amino acids, including Met and cystine (Zheng and Zhang, 1989). The present results demonstrated that dietary supplementation with L-Met increased the length of the fourth wing feather and the moulting score compared to the control birds, indicating that inadequate Met impaired feather growth of broilers. The present findings are similar to those of Zeng et al. (2015) found that dietary supplementation Met improved the feather coverage and the length of the fourth wing feather. Guo et al. (2011) also found that dietary supplementation with Met increased total and relative feather weight and coverage in ducks. This may be related to the fact that Met can promote the deposition of feather protein and improve the sulfur content and keratin composition of feathers. It is noteworthy that L-Met created a better feather trait than DL-Met. The reason may be that the insufficient activitiy of D-AAO in broilers during starter phase, which leads to lack of sulfur containing amino acids for proper feather growth.

The present results with yellow-feathered broilers suggest that Met deficiency served as a stressor, and higher dietary levels of Met did provide an obvious growth promotion effect. The results obtained here using regression analyses, indicate that the Met requirement using either L-Met or DL-Met in medium-speed yellow-feathered broilers are 0.44% and 0.48%, respectively. The higher Met requirement in DL-Met rather than L-Met supplemented birds is due to the lower efficacy of DL-Met compared with L-Met. The stated requirement for Met of Chinese yellow-feathered chicken (Ministry of Agriculture, PRC, 2020) is 0.44% during the start. It phase and the NRC recommended level (Nutrient Requirements of Poultry, National Research Council, 1994) is 0.50%. Thus 0.44% L-Met is recommended for medium-speed yellow-feathered broilers in the starter phase in diets supplemented with L-Met.

Conclusion

Dietary supplementation with L-Met or DL-Met enhanced the growth performance and feather traits of medium-growing yellow-feathered broilers aged 1 to 30 d. The efficiacy of L-Met was higher than DL-Met.



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