

Effects of different methionine sources on production and reproduction performance, egg quality and serum biochemical indices of broiler breeders

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Objective: The study was conducted to evaluate the effects of different methionine (Met) sources on production performance, reproduction performance, egg quality and serum biochemical indices in broiler breeders.

Methods: After receiving a basal diet (containing 0.25% Met) for a 2-wk pretreatment period, a total of 360 39-wk-old Lingnan yellow broiler breeders were randomly allocated to four treatments with six replicates each (15 birds per replicate). Breeders were fed with basal diets (control) or diets supplemented with DL-methionine (DLM), DL-2-hydroxy-4-methylthio butyric calcium (MHA-Ca) and coated DL-Met (CME) respectively.

Results: The results showed that CME supplementation promoted laying rate and decreased feed-to-egg ratio (F/E) ($p < 0.05$), DLM and MHA-Ca supplementation decreased F/E ($p < 0.05$) when compared with control group. The rate of fertility, hatchability and birthrate were higher ($p < 0.05$) in DLM, MHA-Ca, and CME groups than control group. Compared with control group, CME increased the eggshell thickness ($p < 0.05$); MHA-Ca improved the eggshell thickness, shell ratio and eggshell strength ($p < 0.05$). Results also showed that CME elevated the concentration of total protein in serum ($p < 0.05$); MHA-Ca improved the concentration of calcium in serum ($p < 0.05$). The concentration of serum uric acid in DLM, MHA-Ca, and CME groups was lower than that in control group ($p < 0.05$). Besides, CME had higher concentrations of serum taurine, cysteine and cystanthionine ($p < 0.05$) while MHA-Ca and DLM promoted the serum taurine concentration ($p < 0.05$) compared with control group.

Conclusion: Based on the results, it was concluded that Met supplementation could enhance the production and reproduction performance as well as the antioxidant status and egg quality of broiler breeders. In terms of improving the production performance, reproduction performance and antioxidant performance, CME was superior to DLM and MHA-Ca; but in regard to the enhancement of eggshell quality and serum Ca concentration, MHA-Ca was superior to DLM and CME.

Keywords: Broiler Breeder; Methionine; Production Performance; Reproduction Performance; Serum Biochemical Indices

INTRODUCTION

Methionine (Met) plays critical roles in methylation reaction, protein accretion [1], growth performance promotion [2,3] and immune responses enhancement [4]. It is generally considered to be the first limiting amino acid in poultry diets. DL-Met powder (DLM) and DL-2-hydroxy-4-methylthio butanoic acid (MHA) are two major Met sources generally used in feed industry [5]. In recent years, coated DL-Met (CME) has been applied in animal feed because of the high utilization of Met, especially in aquaculture industry [6].

The MHA resembles Met in chemical structure, with a hydroxyl group instead of an amino

group, and it can be equimolar transformed to Met by transamination. Previous studies showed that MHA was equally or less active than DLM in bioavailability [7,8]. However, MHA showed other advantages over DLM, such as lower nitrogen excretion and less toxicity of sulfur-containing amino acid [9]. MHA reacting with calcium carbonate produced DL-2-hydroxy-4-methylthio butyric calcium (MHA-Ca), which can be converted to MHA at an equimolar level. Van [10] reported that MHA-Ca performed higher promotion in egg production than MHA. Coated DL-Met is protected with natural materials which are difficult to degrade by microorganism and sensitive to the change of pH, such as stearic acid, glyceride and gelatin. Those materials help DL-Met effectively to be used in protein synthesis by continuous release in digestive tract [11].

Dietary protein was used in the form of amino acids or small peptides in the process of digestion while free amino acids (FAA) added to the feed were directly absorbed, which leads to low protein synthesis because of the asynchronous absorption. Thus the slower release of FAA in the intestinal tract could give a better utilization. Coating the FAA with a digestion-resistant compound is a good solution. Chi et al [6] reported that compared with the crystalline Met, CME had higher utilization because of the reduced release rate in the intestinal tract. However, no attention has been paid to comparing the effects of DLM, MHA-Ca, and CME on broiler breeders. Thus, the objective of this study was to investigate the effects of different Met sources on production performance, reproduction performance, egg quality and serum biochemical indices in broiler breeders.

MATERIALS AND METHODS

General

This project was approved by the Institution Animal Care and Use Committee at Zhejiang University, and the animal trial was conducted in accordance with the National Institutes of Health Guidelines for the care and use of laboratory animals. DLM was obtained from Evonik (99% pure, Evonik Degussa Co, Dusseldorf, Germany), MHA-Ca was purchased from Novous (containing 84% active substance, Novous Chemical Co, St. Charles City, MO, USA), CME was obtained from King Techina (containing 40% of active substance, King Techina Co, Hangzhou, China).

Experimental design and treatments

A total of 360 39-wk-old *Lingnan* yellow broiler breeders were randomly distributed into four treatments, each of which contained six replicates with 15 birds per replicate. After a 2-wk pretreatment receiving a basal diet shown in Table 1, an 8-wk experiment was conducted. The treatment groups were as follows: i) control group: basal diet, ii) DLM group: basal diet+DLM, iii) MHA-Ca group: basal diet+MHA-Ca, iv) CME group: basal diet+CME. All the Met sources were supplemented in basal diets with an equimolar Met basis, containing 0.1% Met for each experi-

Table 1. Composition and nutrient levels of the basal diets (g/kg, unless otherwise stated)

Items	
Ingredients (%)	
Corn grain	64.0
Soybean meal	26.0
Limestone	5.2
CaHPO ₄	1.0
NaCl	0.3
Shell power	2.5
Premix ¹⁾	1.0
Total	100
Nutrient levels (%)	
ME ²⁾ (MJ/kg)	11.24
Crude protein	15.92
Calcium	3.03
Total phosphorus	0.50
Lysine	0.83
Methionine	0.25
Methionine+cysteine	0.53
Threonine	0.62

ME, metabolizable energy.

¹⁾ Supplied the following per kilogram of diet: iron, 80 mg; copper, 8 mg; zinc, 80 mg; manganese, 100 mg; iodine, 1 mg; selenium, 0.3 mg; vitamin A, 10,800 IU; vitamin D₃, 2,160 IU; vitamin E, 27 mg; vitamin K₃, 1.4 mg; vitamin B₁, 1.8 mg; vitamin B₂, 8 mg; vitamin B₆, 4.1 mg; vitamin B₁₂, 0.01 mg; niacin, 32 mg; D-calcium pantothenate, 11 mg; folic acid, 1.1 mg; biotin, 0.18 mg.

²⁾ ME was calculated from data provide by Feed Database in China [28].

mental group. During the trial period, feed was restricted. All birds were housed in cages with following dimensions (length×depth×front height×rear height): 35.5 cm×35.5 cm×43 cm×50 cm with two hens per cage where the temperature was maintained between 24°C and 30°C. Hens were artificially inseminated weekly. Daily egg production records were kept and summarized per week. All eggs collected were stored in storage room where the temperature was between 10°C and 15°C and incubated once a week, during the incubation period, the number of no-fertilized eggs and dead sperm eggs were recorded to calculate fertility and hatchability of fertile eggs. The amount of live hatched was recorded for the calculation of birthrate. The weight of eggs was recorded daily, and the feed intake was recorded every week to calculate the feed-to-egg ratio (F/E).

Sample collections and preparations

At the end of the experiment, four breeders per replicate were randomly selected and punctured at the wing vein for blood samples with pro-coagulation tubes. Serum was separated by centrifuge at 4°C (3,000 rpm, 10 min), then stored at -80°C for further biochemical analysis.

Chemical analyses

The concentrations of total protein (TP), uric acid (UA), calcium (Ca), and total phosphorus (P) in serum were determined by commercial kits (Nanjing Jiancheng Bio engineering Institute,

Nanjing, China). All the procedures were carried out according to the instructions of the assay kits.

Egg quality determination

Eggshell weights were determined by PL602-L analytical balance (Mettler Toledo Co, Zurich, Switzerland). Eggshell thickness, eggshell strength, albumen height and haugh unit were detected by DET 6,000 egg quality tester (NABEL Co., Ltd, Tokyo, Japan). The length and width, measured by vernier caliper, were recorded to calculate the egg shape index.

Determination of Met metabolites

One mL 2% 5-sulfo salicylic acid and 0.5 mL 2.0 mmol/L ethylenediaminetetraacetic acid were added into 1.0 mL serum samples and them mixed. One hour later, the mixture was centrifuged at 4,000 rpm for 10 min at 4°C, then 5 mL 0.02 mol/L HCl was added into 0.25 mL supernatant. The taurine, cysteine and cystathionine concentrations were determined with L-8900 automatic amino acid analyzer (Hitachi Ltd, Tokyo, Japan) after 0.22 µm polyethylene membrane filtration.

Statistical analysis

All data were statistically analyzed by one-way analysis of variance procedure of SPSS 16.0 for Windows. Comparisons between groups were made by Duncan's multiple range test. Statistical

significance for all data was considered to be $p < 0.05$. The data was presented as means \pm standard deviation.

RESULTS

Production performance

Effects of different Met sources on the production performance of broiler breeders are shown in Table 2. Compared with control group, CME significantly ($p < 0.05$) improved the laying rate while DLM and MHA-Ca showed a trend of promotion ($p > 0.05$). And F/E was significantly ($p < 0.05$) decreased in DLM, MHA-Ca, and CME groups compared with control group. No difference ($p > 0.05$) was observed among the four treatments on average egg weight.

Reproduction performance

Table 3 shows the effects of different Met sources on the reproduction performance of broiler breeders. The supplementation of DLM, MHA-Ca, and CME significantly ($p < 0.05$) improved the fertility rate, hatching rate and birth rate compared with the control group.

Egg quality indices

Effects of different Met sources on egg quality are shown in Table 4. Compared with control group, MHA-Ca enhanced the eggshell thickness, relative eggshell weight and eggshell strength ($p < 0.05$)

Table 2. Effects of different methionine sources on production performance of broiler breeders

Items	Control	DLM	MHA-Ca	CME
Laying rate (%)	50.86 \pm 2.68 ^b	54.52 \pm 1.69 ^{ab}	53.19 \pm 0.75 ^{ab}	55.70 \pm 1.9 ^a
Average egg weight (g)	53.92 \pm 0.64	53.97 \pm 1.12	54.05 \pm 1.25	55.10 \pm 1.21
Feed-to-egg ration(F/E, g:g)	4.63 \pm 0.28 ^a	4.24 \pm 0.13 ^b	4.30 \pm 0.10 ^b	4.29 \pm 0.23 ^b

Control, basal diet; DLM, DL-methionine; MHA-Ca, DL-2-hydroxy-4-methylthio butyric calcium; CME, coated DL-methionine.

^{ab} Different letters significantly different ($p < 0.05$).

Table 3. Effects of different methionine sources on reproduction performance of broiler breeders

Items	Control	DLM	MHA-Ca	CME
Fertility rate (%)	84.11 \pm 2.94 ^b	91.08 \pm 2.88 ^a	90.91 \pm 0.70 ^a	92.09 \pm 1.65 ^a
Hatching rate (%)	86.28 \pm 1.43 ^b	88.09 \pm 2.33 ^a	89.79 \pm 1.73 ^a	90.58 \pm 1.29 ^a
Birth rate (%)	72.54 \pm 1.51 ^b	80.27 \pm 4.70 ^a	81.64 \pm 2.08 ^a	83.43 \pm 2.64 ^a

Control, basal diet; DLM, DL-methionine; MHA-Ca, DL-2-hydroxy-4-methylthio butyric calcium; CME, coated DL-methionine.

^{ab} Different letters significantly different ($p < 0.05$).

Table 4. Effects of different methionine sources on egg quality of broiler breeders

Items	Control	DLM	MHA-Ca	CME
Eggshell thickness (mm)	3.03 \pm 0.02 ^b	3.09 \pm 0.02 ^{ab}	3.23 \pm 0.07 ^a	3.19 \pm 0.10 ^a
Relative eggshell weight (%)	11.33 \pm 0.37 ^b	11.77 \pm 0.35 ^{ab}	12.14 \pm 0.22 ^a	11.88 \pm 0.31 ^{ab}
Eggshell strength (Kgf)	3.14 \pm 0.40 ^b	3.74 \pm 0.06 ^{ab}	4.16 \pm 0.20 ^a	3.69 \pm 0.11 ^{ab}
Egg shape index	0.76 \pm 0.01	0.76 \pm 0.01	0.77 \pm 0.01	0.77 \pm 0.02
Albumen height (mm)	4.09 \pm 0.68	4.00 \pm 0.49	3.99 \pm 0.29	4.37 \pm 0.44
Haugh unit	59.81 \pm 5.82	60.44 \pm 5.03	60.99 \pm 3.04	63.27 \pm 5.44

Control, basal diet; DLM, DL-methionine; MHA-Ca, DL-2-hydroxy-4-methylthio butyric calcium; CME, coated DL-methionine.

^{ab} Different letters significantly different ($p < 0.05$).

while CME significantly ($p < 0.05$) increased the eggshell thickness. No difference ($p > 0.05$) was observed among the groups on the egg shape index, albumen height and haugh unit.

Serum physiological and biochemical indices

The results of different Met sources on serum biochemical profiles are presented in Table 5. Compared with control group, CME elevated the level of TP ($p < 0.05$) and decreased the concentration of UA significantly ($p < 0.05$) in serum. And the UA level of serum also significantly ($p < 0.05$) decreased in DLM, MHA-Ca, and CME groups. The level of serum Ca was higher ($p < 0.05$) in MHA-Ca supplementation group than that of the other three. No difference ($p > 0.05$) was observed among the groups on P level.

Serum Met metabolites concentration

Table 6 shows the effects of different Met sources on serum taurine, cysteine and cystanthionine concentrations. Compared with control group, CME supplementation improved the levels of serum taurine, cysteine and cystanthionine significantly ($p < 0.05$), and DLM and MHA-Ca supplementation significantly ($p < 0.05$) promoted serum taurine concentration.

DISCUSSION

Methionine supplementation in basal diets improved the production performance of broiler breeders. Liu et al [12] reported that 0.02% or 0.04% DL-Met added into basal diet improved broiler breeders' egg weight. Bunchasak and Silapasorn [13] confirmed that Met intake of 439.93 mg/hen/d improved hen-day egg production and egg weight. Our results showed that adding DLM, MHA-Ca, and CME positively affected the laying rate and the F/E of broiler breeders. Moreover, CME significantly improved the laying rate while DLM and MHA-Ca showed a trend of enhancement compared with control group. Meng et al [14] agreed with our studies to a certain extent which they found

that breeders' egg weight, laying rate and feed conversion ratio were significantly increased after taking a Met supplemented diet. The explanation could be that Met supplementation enhances protein deposition, thus promote the egg production. Furthermore, Met supplementation may improve production performance through polyamine metabolism pathways [1].

Researches showed that Met plays a critical role on methylation reactions as an essential intermediate and it can be converted into cysteine, which is engaged into synthesizing glutathione and taurine [15]. Our studies also showed Met has a positive role in promoting the reproduction performance. Compared with control group, the fertility rate, hatchability and birth rate in DLM, MHA-Ca, and CME groups were significantly improved. It may because glutathione and cysteine could clear reactive oxygen species (ROS) directly and hence alleviate the deleterious effects of ROS on lipid, protein and DNA structures [16]. And it appears that excessive ROS generated in the embryo development causes negative impacts, which can result in high embryo mortality [17]. Besides, Bunchasak [13] reported that Met could be transferred to fertile eggs, so Met supplementation could improve the antioxidant performance of chick embryo, which would have a positive effect on embryo development. Therefore, Met supplementation might increase the fertility rate, hatching rate and birthrate.

The eggshell thickness, eggshell strength and relative eggshell weight were directly related to eggs' breakage rate. Bunchasak and Silapasorn [13] reported that adding Met in low crude protein diet was of benefit to hens, particularly on eggshell thickness. Data obtained from our study showed that MHA-Ca supplementation significantly improved the eggshell thickness, relative eggshell weight and eggshell strength with. The reason may because Ca is the first restrictive factor in eggshell formation [18], and the main source of Ca in eggshell are obtained from intestinal absorption and bone Ca mobilization [19]. Thus, the explanation why MHA-Ca promoted the eggshell quality signifi-

Table 5. Effects of different methionine sources on physiological and biochemical indices in serum of broiler breeders

Items	Control	DLM	MHA-Ca	CME
Total protein (TP, g/L)	43.08 ± 4.93 ^b	44.28 ± 2.77 ^b	45.01 ± 3.71 ^{ab}	50.05 ± 6.23 ^a
Uric acid (UA, mg/L)	77.44 ± 13.59 ^a	64.40 ± 6.66 ^b	64.50 ± 9.07 ^b	60.88 ± 6.8 ^b
Calcium (Ca, mmol/L)	2.28 ± 1.02 ^b	2.51 ± 0.13 ^{ab}	3.12 ± 0.99 ^a	2.64 ± 0.42 ^{ab}
Phosphorus (P, mmol/L)	2.05 ± 0.48	2.09 ± 0.71	2.45 ± 0.89	2.38 ± 0.88

Control, basal diet; DLM, DL-methionine; MHA-Ca, DL-2-hydroxy-4-methylthio butyric calcium; CME, coated DL-methionine.

^{ab} Different letters significantly different ($p < 0.05$).

Table 6. Effects of different methionine sources on serum methionine metabolites of broiler breeders

Items	Control	DLM	MHA-Ca	CME
Taurine (mg/L)	36.25 ± 5.88 ^b	48.83 ± 12.30 ^a	48.89 ± 6.46 ^a	49.38 ± 3.29 ^a
Cysteine (mg/L)	18.34 ± 4.27 ^b	19.86 ± 5.49 ^{ab}	20.53 ± 1.89 ^{ab}	24.66 ± 3.78 ^a
Cystanthionine (mg/L)	0.82 ± 0.15 ^b	0.99 ± 0.35 ^{ab}	1.17 ± 0.16 ^{ab}	1.35 ± 0.33 ^a

Control, basal diet; DLM, DL-methionine; MHA-Ca, DL-2-hydroxy-4-methylthio butyric calcium; CME, coated DL-methionine.

^{ab} Different letters significantly different ($p < 0.05$).

cantly may because MHA-Ca provided extra Ca source.

Protein deposition in the body mainly depends on the speed of protein synthesis and catabolism. Serum TP directly reflects the capacity of protein synthesis, and UA is an important indicator of protein catabolism for poultry [20]. It was observed that CME supplementation increased the TP concentration significantly, and DLM, MHA-Ca and CME groups showed a lower serum UA when compared with control group. Results demonstrated that Met could accelerate protein deposition, which was consisted with the laying rate we showed. The explanation maybe that Met could increase the ribosomal capacity ratio (indicator reflecting ribosomal translation efficiency of protein synthesis, up-regulate the mRNA expression levels of protein synthesis-related gene mammalian target of rapamycin (*m-TOR*), S6 Kinase 1 (*S6K1*), and eukaryotic initiation factor 4E (*eIF4E*), as well as down-regulate protein degradation-related genes ubiquitin and cathepsin B [21].

Methionine affected Ca and P metabolism. Richards et al [22] reported that diets with added DLM increased the contents of Ca and P in broiler chickens. Our data also showed that DLM, MHA-Ca, and CME supplementation improved the serum Ca and P levels compared with control group. And the Ca concentration in MHA-Ca was significantly higher than control group, which was consistent with the results of eggshell quality. The possible reason may due to the extra Ca provided.

Cystathionine was generated by transmethylation in the process of Met metabolism [23], and then cystathionine transsulfuration produced cysteine [24], and cysteine finally transformed into taurine [25], which plays important roles in antioxidant [24]. Therefore, Met supplementation could enhance the antioxidant performance of broiler breeders. The present study showed that DLM, MHA-Ca, and CME supplementation increased serum taurine concentration significantly, which consisted with research conducted by Puchala [26], and also agreed with the fertility rate results in our study.

Coated DL-Met is protected with natural materials that are difficult to be degraded by microorganism and sensitive to pH, which help DL-Met effectively utilized by continuous release. This may explain why CME group had a better performance on laying rate and TP. Coated DL-Met allowed Met to be released slowly in the intestine, thus helped free Met added in feed absorbed synchronously with those metabolized from dietary protein [27].

CONCLUSION

In conclusion, the present study demonstrated that DLM, MHA-Ca, and CME supplementation could improve the production reproduction performance and egg quality of broiler breeders. Furthermore, CME showed better performance than DLM and MHA-Ca in enhancing production performance and reproduction performance, while MHA-Ca was superior to DLM and CME in terms of the promotion of eggshell quality and serum Ca concentration.

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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