Postprint: Methionine Requirement of Hy-Line Brown Laying Hens from 25 to 48 Weeks of Age

Authors: Xu Li, Guo Dan, Yue Hongyuan, Zhang Haijun, Wang Jing, Wu Shugeng, Chen Zhengling, Wu Chuanlong

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Abstract

This experiment aimed to investigate the methionine (Met) requirement of Hy-Line Brown laying hens aged 25 to 48 weeks. A total of 720 Hy-Line Brown laying hens with 92% uniformity at 24 weeks of age were selected and randomly divided into 6 groups, with 6 replicates per group and 20 birds per replicate. A corn-soybean meal diet was used in the experiment; the control group received no DL-Met supplementation (Met content 0.25%), while the Met content in the other groups was 0.31%, 0.35%, 0.39%, 0.43%, and 0.47%, respectively. The experimental period lasted 24 weeks. The results showed that: 1) With increasing dietary Met levels, the laying rate and daily egg mass of the hens increased significantly ($P<0.05$), and the feed conversion ratio decreased significantly ($P<0.05$), with the 0.35% and 0.39% Met groups showing better performance; average daily feed intake and average egg weight also improved, but without significant differences ($P>0.05$). 2) Quadratic curve analysis revealed that, based on laying rate, daily egg mass, and feed conversion ratio as evaluation indices, the Met requirement of the laying hens was 0.36% and 0.38% during weeks 1-8 and weeks 9-24 of the experimental period, respectively. 3) At week 4 of the experiment, dietary Met level had no significant effect on plasma total protein (TP) content ($P>0.05$); plasma albumin (ALB) content in the 0.31%, 0.39%, and 0.43% Met groups was significantly higher than that in the control group ($P<0.05$). At week 8, dietary Met level showed no significant effect on plasma TP, ALB, and uric acid (UA) contents ($P>0.05$). At week 24, dietary Met level showed no significant effect on plasma TP and UA contents in laying hens ($P>0.05$). The results suggest that the Met requirement for Hy-Line Brown laying hens aged 25-32 weeks and 33-48 weeks is 0.36% and 0.38%, respectively.

Full Text

Methionine Allowance for Hy-Line Brown Laying Hens Aged 25 to 48 Weeks

GUO Dan[1],[2], YUE Hongyuan[2], ZHANG Haijun[2], *WANG Jing*[2], *XU Li*[1], WU Shugeng[2], CHEN Zhengling[3], WU Chuanlong[3]

[1]College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China
[2]Feed Research Institute, Chinese Academy of Agricultural Sciences/Key Laboratory of Feed Biotechnology, Ministry of Agriculture, Beijing 100081, China
[3]Ningxia Unisplendour Chemical Methionine Co., Ltd., Zhongwei 751700, China

Abstract

This experiment was conducted to investigate the methionine (Met) allowance for Hy-Line Brown laying hens from 25 to 48 weeks of age. A total of 720 healthy Hy-Line Brown laying hens at 24 weeks of age with 92% uniformity were randomly allocated into 6 groups, each consisting of 6 replicates of 20 hens. The experiment utilized corn-soybean meal diets where the control group received no DL-Met supplementation (0.25% Met content), while the remaining groups had dietary Met levels of 0.31%, 0.35%, 0.39%, 0.43%, and 0.47%, respectively. The 24-week trial period revealed several key findings. First, increasing dietary Met levels significantly improved egg-laying rate and daily egg production ($P<0.05$) while significantly reducing feed-to-egg ratio ($P<0.05$), with the 0.35% and 0.39% Met groups showing optimal performance. Average daily feed intake and average egg weight also showed improvement, though these differences were not statistically significant ($P>0.05$). Second, quadratic curve analysis indicated that based on egg-laying rate, daily egg production, and feed-to-egg ratio, the optimal Met allowance was 0.36% for weeks 1-8 and 0.38% for weeks 9-24. Third, at week 4, dietary Met levels did not significantly affect plasma total protein (TP) content ($P>0.05$), but plasma albumin (ALB) content in the 0.31%, 0.39%, and 0.43% Met groups was significantly higher than in the control group ($P<0.05$). At week 8, no significant effects were observed on plasma TP, ALB, or uric acid (UA) levels ($P>0.05$). By week 24, dietary Met levels showed no significant influence on plasma TP or UA content ($P>0.05$). These results suggest that the recommended Met allowance for Hy-Line Brown laying hens is 0.36% for the 25-32 week age period and 0.38% for the 33-48 week age period.

Keywords: DL-methionine; laying hens; performance; blood biochemistry

Introduction

Methionine (Met) is the first limiting amino acid in poultry nutrition and plays a crucial role in growth, production, and health of laying hens. Met supplementation has been shown to increase egg-laying rate and daily egg production while improving performance and egg quality. However, due to the low Met content in plant-based feed ingredients, crystalline Met is commonly supplemented in laying hen diets. Dietary Met supplementation helps balance amino acids, improves protein nutrition and production performance, promotes moderate body weight gain, and reduces feed intake and feed-to-egg ratio. Additionally, Met participates in methyl group transfer and possesses detoxification, anti-reactive oxygen species, and redox balance maintenance functions, while also enhancing immune function. Nevertheless, more is not always better. Multivariate nonlinear modeling has estimated that digestible Met requirements for maximum egg production and optimal feed efficiency are 356 mg/d and 390 mg/d, respectively. Quadratic modeling of 46-53 week-old yellow-feathered broiler breeders indicated Met requirements of 507 mg/d and 494 mg/d for maximum egg production and egg weight, respectively.

Current production practices typically follow NRC (1994) recommendations (0.30%) or national standards (NY/T 33-2004, 0.34%). However, with advancements in poultry breeding and changing nutritional requirements, re-evaluating the needs for the first limiting amino acid holds both theoretical and practical significance. Therefore, this study assessed Met allowance for peak-production laying hens by examining responses in egg-laying rate, daily egg production, feed-to-egg ratio, and average egg weight to varying dietary Met levels.

Materials and Methods

1.1 Met Sample DL-methionine (DLM, 99% purity) was provided by Ningxia Unisplendour Chemical Methionine Co., Ltd.

1.2 Experimental Animals Seven hundred and twenty healthy Hy-Line Brown laying hens at 24 weeks of age with 92% body weight uniformity and normal feed consumption were randomly divided into 6 groups. Each group contained 6 replicates of 20 hens, housed 4 hens per cage (40 cm × 37 cm × 40 cm). No significant differences were observed among groups in body weight, egg-laying rate, or average egg weight ($P<0.05$). The experiment consisted of a 1-week pre-trial period and a 24-week formal trial period, during which hens were gradually transitioned from standard layer feed to experimental diets.

1.3 Experimental Design A single-factor randomized block design was employed. The control group (Group I) received no crystalline DLM supplementation (0.25% Met content), while Groups II-VI had DLM added to achieve dietary Met levels of 0.31%, 0.35%, 0.39%, 0.43%, and 0.47%, respectively. Replicates were distributed evenly throughout the poultry house to minimize environmental effects. Based on NRC (1994), NY/T 33-2004, and the Hy-Line Brown management manual, corn-soybean meal diets were formulated. Dietary composition and nutrient levels are presented in .

1.4 Management Practices Hens were raised in a semi-open three-tier cage system. Each replicate consisted of 5 connected cages, with 4 hens per cage, evenly distributed across 4 rows of stepped cages. Lighting was supplemented morning and evening to maintain a 16L:8D photoperiod at 20 lx intensity. Natural and negative pressure ventilation were combined. Powdered feed was provided ad libitum twice daily at 08:00 and 14:00. Nipple drinkers allowed free access to water. Eggs were collected daily at 13:00 and relevant data recorded.

1.5 Measurements

1.5.1 Production Performance Daily egg number, egg weight, and mortality were recorded per replicate, with feed consumption recorded every two weeks. Egg-laying rate, average daily feed intake (ADFI), feed-to-egg ratio, average egg weight, and daily egg production were calculated for each 2-week interval and the entire experimental period.

1.5.2 Blood Biochemical Indices At the end of weeks 4, 8, and 24, 3 mL of blood was collected from the wing vein using anticoagulant tubes. Plasma was prepared by centrifugation at 3,000 r/min for 10 minutes, and supernatants were stored at -20°C in 1.5 mL Eppendorf tubes. Plasma total protein (TP), albumin (ALB), and uric acid (UA) concentrations were determined using commercial kits from Nanjing Jiancheng Bioengineering Institute on a CHEM-5 semi-automatic biochemical analyzer.

1.6 Statistical Analysis Data were analyzed using one-way ANOVA in SPSS 16.0 software. Duncan' s multiple range test was used for post-hoc comparisons, while t-tests were applied to compare individual treatment groups with the control. Significance was declared at $P<0.05$. Data are expressed as means ± standard deviation. Quadratic curve fitting was performed to determine optimal Met allowance.

Results

2.1 Production Performance Statistical analysis revealed no significant differences in performance during weeks 1-8 ($P>0.05$). Cumulative effects of Met became apparent from week 9 onward; therefore, results are presented for two phases: weeks 1-8 and weeks 9-24.

2.1.1 Performance During Weeks 1-8 As shown in , no significant differences were observed among groups in egg-laying rate, ADFI, feed-to-egg ratio, average egg weight, or daily egg production during weeks 1-8 ($P>0.05$). Groups II, III, and IV had higher egg-laying rates than Group I, though not significantly ($P>0.05$). Group I had the highest ADFI, while Group III had the lowest, with no significant differences among groups ($P>0.05$). Group I showed the highest feed-to-egg ratio, with t-test analysis revealing that Group III had a significantly lower ratio than Group I ($P=0.011$). All treatment groups exhibited higher average egg weight and egg production than Group I, with Group III achieving the highest values, though differences were not significant ($P>0.05$). These results suggest that the Met allowance for Hy-Line Brown laying hens aged 25-32 weeks is approximately 0.35%.

2.1.2 Performance During Weeks 9-24 shows that dietary Met level did not significantly affect egg-laying rate, ADFI, or average egg weight during weeks 9-24 ($P>0.05$). All treatment groups showed improved egg-laying rates compared to Group I, with rates increasing initially then decreasing as Met level rose; Group III achieved the highest rate, significantly exceeding Group I ($P=0.011$). All treatment groups had lower ADFI than Group I, with Group III showing the lowest value, though differences were not significant ($P>0.05$). Feed-to-egg ratio improved in all treatment groups compared to Group I, with Groups III and IV showing 9.87% ($P<0.05$) and 7.30% ($P<0.05$) reductions, respectively. Further increases in Met level tended to elevate the feed-to-egg ratio, demonstrating cumulative Met effects. Dietary Met level did not significantly affect average egg weight ($P>0.05$), though all treatment groups showed improvements, with Group VI achieving the maximum. Daily egg production in Groups III, IV, and V increased by 7.85% ($P<0.05$), 8.52% ($P<0.05$), and 7.73% ($P<0.05$) compared to Group I, respectively, with higher Met levels reducing production. These findings indicate that the Met allowance for Hy-Line Brown laying hens aged 33-48 weeks ranges from 0.35% to 0.39%.

2.1.3 Performance During Weeks 1-24 demonstrates that Met supplementation did not significantly affect egg-laying rate, ADFI, or average egg weight across the entire experimental period ($P>0.05$). All treatment groups showed improved egg-laying rates compared to Group I, with t-test analysis revealing that Group III was significantly higher ($P=0.031$), representing a 5.19% increase. All treatment groups exhibited reduced ADFI compared to Group I. Average egg weight improved with increasing Met levels, with Group V achieving the maximum, though further increases provided no additional benefit. Group III had a significantly lower feed-to-egg ratio than Groups I, II, V, and VI ($P<0.05$), with reductions of 9.32%, 8.55%, 7.76%, and 6.55%, respectively. Daily egg production in Groups III, IV, and V was significantly higher than in Group I ($P<0.05$), with increases of 7.98%, 7.34%, and 6.80%, respectively. These results suggest that the Met allowance for Hy-Line Brown laying hens aged 25-48 weeks ranges from 0.35% to 0.39%.

2.2 Blood Biochemical Indices presents the effects of dietary Met on plasma biochemical indices. At week 4, Met levels did not significantly affect plasma TP content ($P>0.05$), with Group V showing the highest value. Plasma ALB content in Groups II, IV, and V was significantly higher than in Group I ($P<0.05$). Group V had the lowest plasma UA content, while Group VI had the highest. At week 8, dietary Met level did not significantly influence plasma TP, ALB, or UA content ($P>0.05$), with Group III showing the highest TP, Group IV the highest ALB, and Group V the highest UA. At week 24, dietary Met level did not significantly affect plasma TP or UA content ($P>0.05$), though Group V had the lowest ALB content, which was significantly different from Group I ($P<0.05$). Overall, Met levels of 0.35% to 0.43% met the requirements of Hy-Line Brown laying hens aged 25-48 weeks.

2.3 Quadratic Equation Modeling for Peak-Production Met Allowance Although one-way ANOVA indicated that dietary Met level did not significantly affect performance during weeks 1-8 ($P>0.05$), t-test analysis revealed that Group III had a significantly lower feed-to-egg ratio than Group I ($P<0.05$). Therefore, quadratic curve fitting was performed on feed-to-egg ratio data for weeks 1-8.

For weeks 9-24, one-way ANOVA showed that dietary Met level did not significantly affect egg-laying rate, ADFI, or average egg weight ($P>0.05$), but had significant effects on feed-to-egg ratio and daily egg production ($P<0.05$). T-test analysis indicated that Group III had a significantly higher egg-laying rate than Group I ($P<0.05$). Consequently, quadratic curve fitting was applied to egg-laying rate, feed-to-egg ratio, and daily egg production data for this period.

For the entire experimental period (weeks 1-24), one-way ANOVA revealed that dietary Met level did not significantly affect egg-laying rate, ADFI, or average egg weight ($P>0.05$), but significantly influenced feed-to-egg ratio and daily egg production ($P<0.05$). T-test analysis showed that Group III had a significantly higher egg-laying rate than Group I ($P<0.05$). Therefore, quadratic curve fitting was performed on egg-laying rate, feed-to-egg ratio, and daily egg production data for weeks 1-24.

The quadratic equations for each observation indicator across experimental periods are presented in . For weeks 1-8, the quadratic curve fitted to feed-to-egg ratio was significant ($P<0.05$), yielding an optimal Met allowance of 0.36%. Thus, the recommended Met allowance for Hy-Line Brown laying hens aged 25-32 weeks is 0.36%. For weeks 9-24, egg-laying rate, feed-to-egg ratio, and daily egg production all fit quadratic models ($P<0.05$), indicating optimal Met allowances of 0.38%, 0.37%, and 0.39%, respectively. Collectively, the recommended Met allowance for hens aged 33-48 weeks is 0.38%. For the entire period (weeks 1-24), egg-laying rate, feed-to-egg ratio, and daily egg production all conformed to quadratic models ($P<0.05$), with fitted curves indicating Met allowances of 0.37%, 0.37%, and 0.38%, respectively. Overall, the recommended Met allowance for Hy-Line Brown laying hens aged 25-48 weeks is 0.37%.

Discussion

3.1 Effects of Dietary Met Level on Layer Performance As the first limiting amino acid in poultry, Met plays a vital role in poultry production. The present results demonstrate that Group III achieved optimal performance in egg-laying rate, ADFI, feed-to-egg ratio, average egg weight, and daily egg production during weeks 25-32. For weeks 33-48, Met levels of 0.35% to 0.39% produced favorable results based on these performance metrics. Furthermore, egg-laying rate, daily egg production, and average egg weight exhibited a quadratic response to increasing dietary Met levels. Previous research indicates that appropriate Met supplementation can improve egg-laying rate, daily egg production, and average egg weight while reducing ADFI and feed-to-egg ratio, thereby enhancing layer performance, whereas Met deficiency or excess adversely affects performance. In this study, Groups I and VI showed slightly inferior performance compared to other groups, consistent with these findings.

Research suggests that Met levels slightly exceeding NRC (1994) recommendations benefit animal growth. Quadratic modeling in this study yielded optimal Met allowances of 0.36% for weeks 25-32 and 0.38% for weeks 33-48, both higher than NRC (1994) recommendations (0.30%) and national standards (NY/T 33-2004, 0.34%). Studies on Bovans White layers aged 26-34 weeks reported digestible Met requirements of 421 mg/d (summer) and 360 mg/d (winter) for maximum egg production, comparable to our findings for weeks 25-32 ($0.36\% \times 116$ g $= 417$ mg/d). Fisher and Morris estimated Met requirements of 275 mg/d for maximum early-lay egg production using diet dilution techniques. Strathe et al. analyzed 23 trials using multivariate nonlinear mixed-effects models, estimating Met requirements of 356 mg/d for maximum egg production and 390 mg/d for optimal feed efficiency. Lemme analyzed 19 trials and found average Met intake of 415 mg/d at 11.82 MJ/kg energy, exceeding recommendations (390 mg/d) by 6.41%. Joly recommended 420 mg/d Met intake for laying hens. Studies by Liu, Bregendahl, Jais, Coon, Leeson, and Rostagno on ideal amino acid patterns have also reported Met proportions exceeding NRC recommendations, consistent with our results.

3.2 Effects of Dietary Met Level on Blood Biochemical Indices Plasma TP and ALB levels reflect whether dietary protein meets animal requirements and the degree of protein digestion and absorption. Plasma TP also indicates active protein metabolism and reflects amino acid balance and protein utilization efficiency. Higher plasma TP content indicates stronger tissue protein synthesis and promotes organ growth. At week 4, Groups III-V showed relatively high plasma TP and ALB content, indicating good growth status and active tissue protein metabolism conducive to development. At week 8, dietary Met levels of 0.35% and 0.39% produced relatively high plasma TP and ALB content, suggesting optimal growth status. At week 24, plasma TP and ALB content remained relatively high at 0.35% to 0.39% Met, indicating this range met animal requirements and aligned with optimal production performance. As the first limiting amino acid, insufficient Met intake impairs protein synthesis, reduces plasma TP content, and increases UA content, consistent with our findings.

Unused amino acids in the animal body are deaminated in the liver into ammonia and carbon skeletons, with ammonia synthesized into UA or urea nitrogen that enters the bloodstream. UA is the final product of nitrogen metabolism in poultry, and plasma UA accurately reflects protein metabolism and amino acid balance. In this study, plasma UA content at weeks 4, 8, and 24 fell within intermediate ranges at Met levels of 0.35% to 0.43%, indicating high amino acid utilization and adequate Met supply. Miles and Featherston noted that plasma UA has potential value for assessing amino acid requirements. Lower plasma UA content indicates higher protein utilization efficiency, stronger protein anabolism, and more balanced dietary amino acids. Conversely, essential amino acid deficiency or imbalance increases protein catabolism and elevates plasma UA content, consistent with our results.

In conclusion, the recommended Met allowance for Hy-Line Brown laying hens is 0.36% for the 25-32 week age period and 0.38% for the 33-48 week age period.

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Note: Figure translations are in progress. See original paper for figures.