Effects of Methionine Deficiency on Production Performance, Serum Free Amino Acid Content, and Hepatic Methionine Metabolism-Related Gene Expression in Laying Hens during the Late Laying Period (Postprint)

Authors: Liu Yilin, Wu Xin, Yin Yulong, Wang Zhanbin

Date: 2017-10-11T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of methionine deficiency on production performance, serum free amino acid content, and hepatic methionine metabolism-related enzyme gene expression in laying hens during the late laying period. A total of 180 62-week-old Hy-Line Gray laying hens were selected and evenly divided into 3 groups (6 replicates per group, 10 hens per replicate) based on laying rate: the control group was fed a diet with 0.33% methionine level, and the methionine deficiency groups were fed diets with 0.21% and 0.27% methionine levels, respectively, for a 90-day experimental period. The results showed that: 1) Dietary methionine deficiency significantly reduced the average daily feed intake, laying rate, average daily egg weight, average egg weight, and defective egg rate of laying hens ($P<0.05$), and significantly increased the feed-to-egg ratio ($P<0.05$). 2) Methionine deficiency significantly decreased the methionine content in serum of laying hens ($P<0.05$). The contents of serine, glycine, and alanine in serum of laying hens in the 0.21% methionine group were significantly higher than those in the other two groups ($P<0.05$). The contents of valine, isoleucine, and arginine in serum of laying hens in the 0.27% methionine group were significantly lower than those in the control group ($P<0.05$), and the proline content was significantly lower than that in the 0.21% methionine group ($P<0.05$). 3) Compared with the control group, the expression levels of DNA methyltransferase 1, N6-methyladenosine (m6A) methyltransferase 3 (METTL3), and m6A methyltransferase 14 in the liver of laying hens in the 0.21% methionine group were significantly increased ($P<0.05$), and the expression level of METTL3 in the liver of laying hens in the 0.27% methionine group was significantly increased

(P<0.05). 4) Compared with the control group, the expression levels of methionine adenosyltransferase 1a, 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), and cystathionine-β-synthase in the liver of laying hens in the 0.21% methionine group were significantly increased (P<0.05), and the expression level of MTR in the liver of laying hens in the 0.27% methionine group was significantly increased (P<0.05). Methionine deficiency had no significant effect on the expression levels of methionine adenosylhomocysteinase and betaine-homocysteine methyltransferase in the liver of laying hens (P>0.05). Based on the above results, it can be concluded that high-level methionine deficiency in the diet of laying hens reduces production performance, which may be related to methionine deficiency altering methionine metabolic pathways and affecting DNA and RNA methylation processes.

Full Text

Effects of Methionine Deficiency on Performance, Serum Free Amino Acid Contents, and Liver Methionine Metabolism-Related Gene Expression in Laying Hens during the Late Laying Period

LIU Yilin[1], WU Xin[2], YIN Yulong[1,2], WANG Zhanbin[1]*

[1]Henan Provincial Academician Workstation of Feed Resource Development and Healthy Livestock, College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471003, China
[2]Hunan Provincial Engineering Research Center of Healthy Livestock, Key Laboratory of Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

Abstract: This experiment was conducted to investigate the effects of dietary methionine deficiency on production performance, serum free amino acid contents, and hepatic methionine metabolism-related gene expression in laying hens during the late laying period. A total of 180 Hy-Line Grey laying hens aged 62 weeks were allocated to three groups (six replicates per group, ten hens per replicate) based on uniform egg production rate. The control group received a diet containing 0.33% methionine, while the methionine-deficient groups received diets containing 0.21% and 0.27% methionine, respectively. The trial lasted for 90 days. The results showed that: (1) Dietary methionine deficiency significantly reduced average daily feed intake, laying rate, average daily egg production, average egg weight, and unqualified egg rate ($P < 0.05$), while significantly increasing the feed-to-egg ratio ($P < 0.05$). (2) Methionine deficiency significantly decreased serum methionine concentration ($P < 0.05$). The 0.21% methionine group exhibited significantly higher serum serine, glycine, and alanine contents compared with the other two groups ($P < 0.05$). The 0.27% methionine group showed significantly lower serum valine, isoleucine, and arginine contents than the control group ($P < 0.05$), and significantly lower proline content

than the 0.21% methionine group ($P < 0.05$). (3) Compared with the control group, the 0.21% methionine group demonstrated significantly elevated hepatic expression of DNA methyltransferase 1, N6-adenosine-methyltransferase subunit 3 (METTL3), and N6-adenosine-methyltransferase subunit 14 (METTL14) ($P < 0.05$), while the 0.27% methionine group also showed significantly increased METTL3 expression ($P < 0.05$). (4) The 0.21% methionine group exhibited significantly higher hepatic expression of methionine adenosyltransferase 1a, cystathionine-β-synthase, and 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) compared with the control group ($P < 0.05$), and the 0.27% methionine group also showed significantly increased MTR expression ($P < 0.05$). Methionine deficiency had no significant effect on the expression of adenosylhomocysteinase or betaine-homocysteine methyltransferase ($P > 0.05$). In conclusion, high-level methionine deficiency in laying hen diets impairs production performance, possibly by altering methionine metabolic pathways and affecting DNA and RNA methylation processes.

Keywords: methionine; laying hens; performance; methionine metabolism; methylation; gene expression

---

Methionine is the only sulfur-containing essential amino acid that participates in protein synthesis and serves as a precursor for cysteine, reduced glutathione, and taurine synthesis. It also provides methyl groups for transmethylation reactions in the synthesis of creatine, phosphatidylcholine, and polyamines, as well as for DNA, RNA, and histone methylation. Metabolic adaptation to methionine restriction can enhance mitochondrial biogenesis and function, increase energy expenditure, alter lipid and carbohydrate homeostasis, reduce oxidative damage and inflammation, and extend lifespan. However, methionine is the first limiting amino acid in laying hens, and restricting its dietary supplementation can decrease egg production rate, egg weight, feed utilization, and body weight while causing hepatic lipid accumulation. These effects stem not only from impaired amino acid balance and protein utilization but also from altered methionine metabolism, including changes in metabolic intermediates such as glycine, serine, choline, cysteine, and glutathione. Nevertheless, the specific effects of methionine deficiency on methionine metabolic pathways remain unreported. This study aimed to investigate the effects of dietary methionine deficiency on production performance, serum free amino acid contents, and methionine metabolism-related enzyme gene expression in Hy-Line Grey laying hens, thereby providing a theoretical basis for the scientific utilization of methionine.

1.1 Experimental Design

This experiment employed a single-factor completely randomized block design. A total of 180 Hy-Line Grey laying hens aged 62 weeks were divided into three groups with uniform egg production rates, each consisting of six replicates of

ten hens. The control group received a basal diet containing 0.33% methionine, while the methionine-deficient groups received diets containing 0.21% and 0.27% methionine, respectively. The basal diet composition and nutrient levels are presented in . Hens were housed in three-tier cages (two hens per cage) with each replicate randomly distributed throughout the poultry house and provided with nipple drinkers. Lighting was supplemented at night to maintain a total photoperiod of 16 h daily. Feed and water were provided ad libitum, with feeding at 07:30 and 15:30 each day. The experimental period lasted 90 days.

1.2 Experimental Materials

DL-methionine (99% purity) was purchased from Adisseo France S.A.S.

1.3 Measurements

1.3.1 Production Performance

Feed intake, daily egg number, broken/misshapen eggs, egg weight, and broken egg weight were recorded weekly by replicate. Laying rate, average daily egg production, average daily feed intake, feed-to-egg ratio, average egg weight, and unqualified egg rate were calculated accordingly.

1.3.2 Serum Free Amino Acid Contents

On day 90, six hens per group were randomly selected for blood collection via jugular vein puncture. Serum was prepared by centrifugation at 3,000 r/min for 10 min and stored at -20°C. For analysis, 600 µL of serum was mixed with 600 mL of 8% sulfosalicylic acid, vortexed, and left overnight at 4°C. After centrifugation at 8,000 r/min for 10 min, the supernatant was filtered through a 0.22 µm filter, and 500 µL of the filtrate was analyzed using an amino acid analyzer.

1.3.3 Hepatic Methionine Metabolism-Related Gene Expression

Following slaughter, livers were rapidly excised, snap-frozen in liquid nitrogen, and stored at -80°C for real-time quantitative PCR analysis. The 10 µL PCR reaction mixture contained 1 µL cDNA template, 5 µL SYBR Green fluorescent dye, 0.3 µL each of forward and reverse primers, and 3.4 µL double-distilled water. Target genes included methionine adenosyltransferase 1a (MAT1a), DNA methyltransferase 1 (Dnmt1), DNA methyltransferase 3a (Dnmt3a), N6-adenosine-methyltransferase subunit 3 (METTL3), N6-adenosine-methyltransferase subunit 14 (METTL14), adenosylhomocysteinase (Ahcy), 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), betaine-homocysteine methyltransferase (BHMT), and cystathionine-β-synthase (CBS). Primers were designed based on chicken gene sequences using NCBI ().

1.4 Statistical Analysis

Data are expressed as means and standard errors, with $P < 0.05$ considered statistically significant. The $2^{-\Delta\Delta C_t}$ method was used to calculate relative gene expression levels using β-actin as the internal reference gene. Experimental

data were analyzed using one-way ANOVA in SPSS 17.0, followed by Duncan' s multiple comparison test.

2.1 Effects of Methionine Deficiency on Production Performance

As shown in , dietary methionine level significantly affected all production performance parameters ($P < 0.05$). Laying rate, average daily egg production, and average egg weight increased significantly with higher dietary methionine levels ($P < 0.05$). The 0.33% methionine group (control) exhibited significantly higher average daily feed intake and unqualified egg rate than both the 0.21% and 0.27% methionine groups ($P < 0.05$). The feed-to-egg ratio in the 0.21% methionine group was significantly higher than that in the 0.27% and 0.33% methionine groups ($P < 0.05$).

2.2 Effects of Methionine Deficiency on Serum Free Amino Acid Contents

Methionine deficiency significantly affected serum methionine concentration, which decreased markedly with lower dietary methionine levels ($P < 0.05$). The 0.21% methionine group showed significantly higher serum serine, glycine, and alanine contents than the other two groups ($P < 0.05$). Serum valine, isoleucine, and arginine contents in the 0.27% methionine group were significantly lower than those in the control group ($P < 0.05$), while serum proline content was significantly lower than that in the 0.21% methionine group ($P < 0.05$).

2.3 Effects of Methionine Deficiency on Hepatic Methionine Metabolism-Related Gene Expression

Dietary methionine level significantly influenced the hepatic expression of MAT1a, Dnmt1, METTL3, METTL14, CBS, and MTR ($P < 0.05$), but had no significant effect on Dnmt3a, Ahcy, or BHMT expression ($P > 0.05$). The 0.21% methionine group exhibited significantly higher hepatic expression of Dnmt1, METTL3, and METTL14 compared with the other two groups ($P < 0.05$), while the 0.27% methionine group showed significantly increased METTL3 expression relative to the control group ($P < 0.05$). Additionally, the 0.21% methionine group demonstrated significantly elevated hepatic expression of MAT1a and MTR compared with both other groups ($P < 0.05$), and significantly increased CBS expression relative to the control group ($P < 0.05$). The 0.27% methionine group also showed significantly higher MTR expression than the control group ($P < 0.05$).

3.1 Effects of Methionine Deficiency on Production Performance

Methionine is the first limiting amino acid for laying hens and plays a crucial role in their production performance. Previous research indicates that increasing methionine levels can improve performance when methionine content is low, but excessive supplementation may reduce performance once optimal levels are

reached. In this study, all dietary methionine levels were relatively low, and the 0.33% methionine group achieved the highest performance metrics, though the feed-to-egg ratio did not differ significantly from the 0.27% methionine group. Saki et al. reported that increasing methionine from 0.24% to 0.34% significantly improved laying rate, average egg weight, average daily egg production, and average daily feed intake while reducing the feed-to-egg ratio. Harms et al. found that dietary methionine levels (0.20%-0.34%) significantly affected laying rate, average egg weight, and average daily feed intake, with no further significant effects observed beyond 0.28% methionine. However, Keshavarz demonstrated that reducing methionine from 0.36% to 0.23% in 54-72-week-old Single Comb White Leghorn hens significantly decreased laying rate, average egg weight, and feed efficiency, but did not significantly affect average daily egg production or average daily feed intake.

3.2 Effects of Dietary Methionine Deficiency on Serum Free Amino Acid Contents

Serum free amino acid concentrations reflect amino acid metabolism status in animals. Due to synergistic, alternative, conversion, and antagonistic relationships among amino acids, deficiency or excess of any particular amino acid can disrupt amino acid ratios and affect utilization of itself or other amino acids. This study demonstrated that dietary methionine level significantly influenced the content and ratio of certain serum amino acids, decreasing methionine, cysteine, isoleucine, and arginine while increasing serine and glycine. Consistent with these findings, Lü et al. reported that dietary methionine level affected serine, methionine, and isoleucine contents. Similarly, Yodseranee et al. observed that dietary methionine level influenced plasma methionine, cysteine, and taurine concentrations in broilers.

3.3 Effects of Dietary Methionine Deficiency on Hepatic Methionine Metabolism

Approximately 20% of ingested methionine is metabolized in the gastrointestinal tract, while 80% is transported via blood to cells and tissues, with over half being converted to S-adenosylmethionine in the liver to enter methionine metabolic pathways. Methionine is converted to S-adenosylmethionine through ATP consumption by methionine adenosyltransferase, which is primarily encoded by MAT1a. More than 90% of S-adenosylmethionine is subsequently converted to S-adenosylhomocysteine by methyltransferases, with the released methyl groups participating in DNA and RNA methylation processes to regulate gene expression and RNA-mediated cellular pathways. In this study, methionine deficiency significantly affected Dnmt1, METTL3, and METTL14 expression, suggesting that methionine deficiency may influence DNA and RNA methylation. Mattocks et al. found that short-term methionine restriction improved the efficiency of DNA methylation maintenance systems and increased global hepatic DNA methylation in adult mice. Conversely, Liu et al. reported that low- and high-

methionine groups showed 46% and 83% methylation levels, respectively, in a GC-rich region of the myostatin gene exon 1, and that either excessive or insufficient methionine intake could cause demethylation when GC-rich regions were already hypermethylated.

RNA methylation represents the most common and abundant RNA modification, with m6A formed on carbon or nitrogen atoms using methyl groups from S-adenosylmethionine. METTL3 is the active component of the m6A methyltransferase complex in mammalian cells, and METTL3 knockout can decrease total m6A levels by 30% in HeLa cells and induce apoptosis in HepG2 cells via p53-mediated pathways. METTL14 is another active component of the m6A methyltransferase complex that forms a stable heterodimeric complex with METTL3 at a 1:1 stoichiometric ratio to regulate m6A deposition on mRNA. While no previous studies have reported the effects of methionine deficiency on RNA methylation, our results showing significantly increased METTL3 and METTL14 expression suggest that methionine deficiency may affect RNA methylation levels in a dose-dependent manner.

S-adenosylhomocysteine is decomposed to adenosine and homocysteine by Ahcy in a reversible, non-rate-limiting step whose metabolic flux is determined by synthesis rates of S-adenosylhomocysteine and consumption rates of adenosine and homocysteine. The lack of significant differences in Ahcy expression among the three groups suggests that methionine deficiency may not affect the ratio of these metabolites. Homocysteine is subsequently metabolized through two pathways: (1) remethylation to methionine by MTR using 5-methyltetrahydrofolate or by BHMT using betaine as methyl donors, constituting the methionine cycle; or (2) transsulfuration to cystathionine catalyzed by CBS with serine participation, followed by involvement in cysteine, glutathione, or taurine synthesis and oxidative degradation. The tetrahydrofolate demethylated in this process can be regenerated to 5-methyltetrahydrofolate through the folate cycle, concurrently converting serine to glycine. The significant effects of methionine deficiency on MAT1a, CBS, and MTR expression indicate altered homocysteine metabolism. Furthermore, decreased serum cysteine and significant accumulation of serine and glycine in the high methionine deficiency group suggest that methionine deficiency may reduce transsulfuration while enhancing remethylation, with the reduction in transsulfuration potentially exceeding the increase in remethylation. Research indicates that during methionine deficiency, homocysteine remethylation to methionine ensures normal methylation reactions, albeit at the cost of reduced α-ketobutyrate and glutathione synthesis. Conversely, a mere 10% increase in methionine levels can double homocysteine synthesis efficiency. Methionine loading studies have shown that methionine overload activates homocysteine transsulfuration while inhibiting remethylation in rat hepatocytes, significantly reducing BHMT and methionine synthase activities without affecting CBS activity. However, our study found that methionine deficiency also significantly increased CBS expression. Tang et al. demonstrated that methionine deprivation induces tissue-specific downregulation of CBS protein through an S-adenosylmethionine-independent mechanism to efficiently conserve methionine,

while mRNA levels remained unchanged or even increased, consistent with our findings.

In conclusion, methionine deficiency significantly reduced laying rate, average daily egg production, and average egg weight while increasing the feed-to-egg ratio in Hy-Line Grey laying hens during the late laying period. Methionine deficiency significantly increased hepatic expression of Dnmt1, METTL3, and METTL14, affecting DNA and RNA methylation processes. Additionally, methionine deficiency significantly decreased serum methionine content, increased serum glycine and serine contents, and elevated hepatic MTR expression, thereby influencing methionine resynthesis and transsulfuration pathways.

References:

[1] AKBARI MOGHADDAM KAKHKI R, GOLIAN A, ZARGHI H. Effect of digestible methionine + cystine concentration on performance, egg quality and blood metabolites in laying hens[J]. British Poultry Science, 2016, 57(3): 403-414.

[2] BERTOLO R F, MCBREAIRTY L E. The nutritional burden of methylation reactions[J]. Current Opinion in Clinical Nutrition and Metabolic Care, 2013, 16(1): 102-108.

[3] PERRONE C E, MALLOY V L, ORENTREICH D S, et al. Metabolic adaptations to methionine restriction that benefit health and lifespan in rodents[J]. Experimental Gerontology, 2013, 48(7): 654-666.

[4] KIKUSATO M, SUDO S, TOYOMIZU M. Methionine deficiency leads to hepatic fat accretion via impairment of fatty acid import by carnitine palmitoyltransferase I[J]. British Poultry Science, 2015, 56(2): 225-231.

[5] JANKOWSKI J, KUBIŃSKA M, ZDUŃCZYK Z. Nutritional and immunomodulatory function of methionine in poultry diets-a review[J]. Annals of Animal Science, 2014, 14(1): 17-31.

[6] MATTOCKS D A L, MENTCH S J, SHNEYDER J, et al. Short term methionine restriction increases hepatic global DNA methylation in adult but not young male C57BL/6J mice[J]. Experimental Gerontology, 2017, 88: 1-8.

[7] 梁中军, 王瑛, 韩雪娇, 等. 饲粮蛋氨酸水平对蛋鸡产蛋后期生产性能的影响 [J]. 动物营养学报, 2015, 27(9): 2699-2704.

[8] SAKI A A, NASERI HARSINI R, TABATABAEI M M, et al. Estimates of methionine and sulfur amino acid requirements for laying hens using different models[J]. Revista Brasileira de Ciência Avícola, 2012, 14(3): 209-216.

[9] HARMS R H, RUSSELL G B. Performance of commercial laying hens fed diets with various levels of methionine[J]. The Journal of Applied Poultry Research, 2003, 12(4): 449-455.

[10] KESHAVARZ K. Effects of reducing dietary protein, methionine, choline, folic acid, and vitamin B12 during the late stages of the egg production cycle on

performance and eggshell quality[J]. Poultry Science, 2003, 82(9): 1407-1414.

[11] 吕明斌, 孙作为, 燕磊, 等. 肉仔鸡饲粮中蛋氨酸和半胱氨酸与赖氨酸适宜比例的研究 [J]. 动物营养学报, 2011, 23(12): 2109-2117.

[12] YODSERANEE R, BUNCHASAK C. Effects of dietary methionine source on productive performance, blood chemical, and hematological profiles in broiler chickens under tropical conditions[J]. Tropical Animal Health and Production, 2012, 44(8): 1957-1963.

[13] ROBINSON J L, MCBREAIRTY L E, RANDELL E W, et al. Restriction of dietary methyl donors limits methionine availability and affects the partitioning of dietary methionine for creatine and phosphatidylcholine synthesis in the neonatal piglet[J]. The Journal of Nutritional Biochemistry, 2016, 35: 81-86.

[14] MATO J M, LU S C. Role of S-adenosyl-L-methionine in liver health and injury[J]. Hepatology, 2007, 45(5): 1306-1312.

[15] WATERLAND R A. Assessing the effects of high methionine intake on DNA methylation[J]. The Journal of Nutrition, 2006, 136(6S): 1706S-1710S.

[16] JIA G F, FU Y, HE C. Reversible RNA adenosine methylation in biological regulation[J]. Trends in Genetics, 2013, 29(2): 108-115.

[17] FINKELSTEIN J D. The metabolism of homocysteine: pathways and regulation[J]. European Journal of Pediatrics, 1998, 157(Suppl 2): S40-S44.

[18] LIU G Q, ZONG K, ZHANG L L, et al. Dietary methionine affects meat quality and myostatin gene exon 1 region methylation in skeletal muscle tissues of broilers[J]. Agricultural Sciences in China, 2010, 9(9): 1338-1346.

[19] NIU Y M, ZHAO X, WU Y S, et al. N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function[J]. Genomics, Proteomics & Bioinformatics, 2013, 11(1): 8-17.

[20] FU Y, DOMINISSINI D, RECHAVI G, et al. Gene expression regulation mediated through reversible m6A RNA methylation[J]. Nature Reviews Genetics, 2014, 15(5): 293-306.

[21] LIU J Z, YUE Y N, HAN D L, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation[J]. Nature Chemical Biology, 2014, 10(2): 93-95.

[22] MARTINOV M V, VITVITSKY V M, MOSHAROV E V, et al. A substrate switch: a new mode of regulation in the methionine metabolic pathway[J]. Journal of Theoretical Biology, 2000, 204(4): 521-532.

[23] BROSNAN J T, BROSNAN M E. The sulfur-containing amino acids: an overview[J]. The Journal of Nutrition, 2006, 136(6): 1636S-1640S.

[24] PILLAI P B, FANATICO A C, BEERS K W, et al. Homocysteine remethylation in young broilers fed varying levels of methionine, choline, and betaine[J]. Poultry Science, 2006, 85(1): 90-95.

[25] MATO J M, LU S C. The hepatocarcinogenic effect of methionine and choline deficient diets: an adaptation to the Warburg effect?[J]. Alcoholism: Clinical and Experimental Research, 2011, 35(5): 811–814.

[26] 高蔚娜, 韩超, 韦京豫, 等. 蛋氨酸负载对大鼠肝细胞 Hcy 代谢酶及相关代谢物的影响 [J]. 营养学报, 2013, 35(6): 545–548.

[27] TANG B Q, MUSTAFA A, GUPTA S, et al. Methionine-deficient diet induces post-transcriptional downregulation of cystathionine β-synthase[J]. Nutrition, 2010, 26(11/12): 1170–1175.

Corresponding author, professor, E-mail: wangzhanbin3696@126.com

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