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

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

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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, while the methionine deficiency groups were fed diets with 0.21% and 0.27% methionine, respectively, for a 90-day experimental period. The results showed that: 1) Dietary methionine deficiency significantly reduced average daily feed intake, laying rate, average daily egg weight, average egg weight, and defective egg rate ($P<0.05$), and significantly increased feed-to-egg ratio ($P<0.05$). 2) Methionine deficiency significantly decreased serum methionine content in laying hens ($P<0.05$). The serum contents of serine, glycine, and alanine in the 0.21% methionine group were significantly higher than those in the other two groups ($P<0.05$). The serum contents of valine, isoleucine, and arginine in the 0.27% methionine group were significantly lower than those in the control group ($P<0.05$), and 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 (DNMT1), N6-methyladenosine (m6A) methyltransferase 3 (METTL3), and m6A methyltransferase 14 (METTL14) 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 (MAT1A), 5-methyltetrahydrofolate-homocysteine methyltrans-

ferase (MTR), and cystathionine- -synthase (CBS) 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 (AHCY) and betaine-homocysteine methyltransferase (BHMT) in the liver of laying hens (P>0.05). Based on these results, it can be concluded that high-level methionine deficiency in laying hen diets 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 Gene Expression of Laying Hens during Late Period of Laying

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 methionine deficiency on production performance, serum free amino acid contents, and liver methionine metabolism-related enzyme gene expression in laying hens during the late laying period. A total of 180 Hy-Line Grey laying hens aged 62 weeks were selected and divided into three groups (six replicates per group, ten hens per replicate) based on uniform egg production rate. The control group was fed a diet containing 0.33% methionine, while the methionine-deficient groups were fed diets containing 0.21% and 0.27% methionine, respectively. The experimental period lasted 90 days.

The results showed: (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 content (P<0.05). The serum contents of serine, glycine, and alanine in the 0.21% methionine group were significantly higher than those in the other two groups (P<0.05). The serum contents of valine, isoleucine, and arginine in the 0.27% methionine group were significantly lower than those

in the control group (P<0.05), and serum 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 hens in the 0.21% methionine group were significantly increased (P<0.05), and the METTL3 expression level in the liver of hens in the 0.27% methionine group was also 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- -synthase in the liver of hens in the 0.21% methionine group were significantly increased (P<0.05), and the MTR expression level in the liver of hens in the 0.27% methionine group was significantly increased (P<0.05). Methionine deficiency had no significant effect on the expression levels of adenosylhomocysteinase and betaine-homocysteine methyltransferase in the liver of hens (P>0.05).

In conclusion, high-level methionine deficiency in laying hen diets reduces production performance, which may be related to altered methionine metabolic pathways and affected DNA and RNA methylation processes.

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

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Methionine is the only sulfur-containing essential amino acid that participates in protein synthesis in the body. It serves as a precursor for cysteine, reduced glutathione, and taurine synthesis, and provides methyl groups for transmethylation reactions in creatine, phosphatidylcholine, and polyamine synthesis, as well as for DNA, RNA, and histone methylation. The body can improve mitochondrial biogenesis and function, energy expenditure, lipid and carbohydrate homeostasis, reduce oxidative damage and inflammation, and extend lifespan through metabolic adaptation to methionine restriction. However, methionine is the first limiting amino acid for laying hens, and restricting its dietary supplementation can cause decreased egg production rate, egg weight, feed utilization, body weight, and hepatic lipid accumulation. This occurs not only because methionine deficiency affects amino acid balance and protein utilization, but also due to alterations in methionine metabolism, such as changes in the levels of metabolic intermediates including glycine, serine, choline, cysteine, and glutathione. Nevertheless, the specific effects of methionine deficiency on the methionine metabolism process have not been reported. This study aimed to use Hy-Line Grey laying hens as experimental animals to investigate the effects of dietary methionine deficiency on production performance, serum free amino acid contents, and methionine metabolism-related enzyme gene expression, 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 selected and divided into three groups based on uniform egg production rate, with six replicates per group and ten hens per replicate. The control group was fed a diet containing 0.33% methionine (basal diet), while the methionine-deficient groups were fed diets containing 0.21% and 0.27% methionine, respectively. The composition and nutrient levels of the basal diet are shown in Table 1 . The laying hens were housed in three-tier cages, with two hens per cage, and each replicate was randomly distributed throughout the poultry house with nipple drinkers. Supplemental lighting was provided at night, with a total daily light duration of 16 hours. The hens had free access to feed and water, with feeding at 07:30 and 15:30 daily. The experimental period lasted 90 days.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Ingredients	Content	Nutrient levels[2])
Corn	-	Metabolizable energy ME/(MJ/kg)
Soybean meal	-	Crude protein CP
Expanded soybean	-	Calcium Ca
Wheat bran	-	Total phosphorus TP
Limestone	-	Available phosphorus AP
CaHPO	-	Methionine Met
Choline chloride	-	Methionine+Cysteine Met+Cys
NaCl	-	Lysine Lys
Premix[1])	-	-
Zeolite powder	-	-
Total	-	-

[1]) The premix provided the following per kg of the diet: VA 12,000 IU, VB 3 mg, VB 9 mg, VB 6 mg, VB 0.03 mg, VD 3,000 IU, VE 30 IU, VK 6 mg, pantothenate 18 mg, biotin 0.12 mg, folic acid 1.5 mg, nicotinamide 6 mg, Mn 106 mg, I 0.8 mg, Fe 90 mg, Cu 6.4 mg, Zn 70 mg, Se 0.3 mg.
[2]) Nutrient levels were calculated values.

1.2 Experimental Materials

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

1.3.1 Production Performance

During the experimental period, weekly feed intake, daily egg number, broken/misshapen eggs, egg weight, and broken egg weight were recorded on a replicate basis. Laying rate, average daily egg production, average daily feed

intake, feed-to-egg ratio, average egg weight, and unqualified egg rate were calculated.

1.3.2 Serum Free Amino Acid Contents

On day 90 of the experiment, six laying hens were randomly selected from each group, and blood was collected from the jugular vein. Serum was prepared by centrifugation at 3,000 r/min for 10 minutes 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 minutes, the supernatant was filtered through a 0.22 µm filter, and 500 µL of the filtrate was analyzed for free amino acid content using an amino acid analyzer.

1.3.3 Expression of Methionine Metabolism-Related Enzyme Genes

After the hens were slaughtered, the liver was quickly removed and snap-frozen in liquid nitrogen, then stored at -80°C for real-time fluorescent quantitative PCR detection. The real-time fluorescent quantitative PCR was performed in a 10 µL system containing 1 µL cDNA template, 5 µL SYBR Green fluorescent dye, 0.3 µL forward primer, 0.3 µL reverse primer, and 3.4 µL double-distilled water. The genes measured included methionine adenosyltransferase 1a (MAT1a), DNA methyltransferase 1 (Dnmt1), DNA methyltransferase 3a (Dnmt3a), N6-methyladenosine (m6A) methyltransferase 3 (METTL3), m6A methyltransferase 14 (METTL14), adenosylhomocysteinase (Ahcy), 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), betaine-homocysteine methyltransferase (BHMT), and cystathionine- -synthase (CBS). Primers were designed based on chicken gene sequences using NCBI (Table 2).

Table 2 Primer sequences

Genes	Primer sequences (5 –3)	Product size/bp
-actin	F:TTACTCGCCTCTGTGAAGGC R:TCCTAGACTGTGGGGGACTG	-
MAT1a	F:TCGTCGTGTTCTGGTTCAGG R:GACAATGACTCCAGGCCGAA	-
Dnmt1	F:CAAGATCGAGACCACCGTCC R:GTCCTTGTCGATCCTGGTGG	-
Dnmt3a	F:AGTTCTCAGTGGTCTGCGTG R:CCACCTTGGAGGTGTCAGTC	-
METTL3	F:CTACGAACGCGTGGATGAGA R:AGAGTTCGATCTTCCGCGTG	-
METTL14	F:ACCCCAAGGCTGTTTTCCAA R:TCGTCCAAGGCAGAAATGCT	-
Ahcy	F:GCCCTTTGCCATCATCCTCT R:TACTGGGACATTAGGGGCCA	-
CBS	F:ACGCATGCTAATCCGAGAGG R:AGTTGGAAGCACAGTCAGGG	-
MTR	F:GGCTCTTGGAGATCGACTGG R:CGAGCTTCCACATGGTGAGT	-
BHMT	F:GCCTGAAACAGGGCAAAAGG R:TCCCTGTGAAGCTGACGAAC	-

1.4 Statistical Analysis

Data were expressed as means and standard errors, with P<0.05 considered statistically significant. The -actin gene was used as an internal reference, and the 2^(-ΔΔCt) method was used to calculate relative gene expression levels. Experimental data were analyzed using one-way ANOVA with SPSS 17.0, and Duncan' s multiple range test was used for post-hoc comparisons.

2.1 Effects of Methionine Deficiency on Production Performance of Laying Hens

As shown in Table 3 , dietary methionine level significantly affected all production performance indicators (P<0.05). Laying rate, average daily egg production, and average egg weight increased significantly with increasing dietary methionine level (P<0.05). Average daily feed intake and unqualified egg rate in the 0.33% methionine group (control) were significantly higher than those in 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).

Table 3 Effects of methionine deficiency on performance of laying hens

Items	Dietary methionine level/%	P-value
	0.21%	0.27%
Average daily feed intake (ADFI)/(g/d)	96.85c	101.30b
Egg-laying rate/%	76.65c	81.02b
Feed/egg	2.22a	2.09b
Average daily egg production/(g/d)	43.65c	48.45b
Average egg weight/g	56.98c	59.85b
Unqualified rate of eggs/%	0.05b	0.10b

In the same row, values with no letter or the same letter superscripts mean no significant difference (P>0.05), while different small letter superscripts mean significant difference (P<0.05). The same as below.

2.2 Effects of Methionine Deficiency on Serum Free Amino Acid Contents of Laying Hens

As shown in Table 4 , methionine deficiency significantly affected serum methionine content, which decreased significantly with decreasing dietary methionine level (P<0.05). Serum serine, glycine, and alanine contents in the 0.21% methionine group were significantly higher than those in 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 0.21% and 0.33% methionine groups (P<0.05), and serum proline content was significantly lower than that in the 0.21% methionine group (P<0.05).

Table 4 Effect of methionine deficiency on serum free amino acid contents of laying hens (μg/mL)

Items	Dietary methionine level/%	P-value
	0.21%	0.27%
Methionine (Met)	8.65c	14.80a
Cysteine (Cys)	98.41a	78.69b
Serine (Ser)	44.73a	39.79b
Glycine (Gly)	57.78a	45.29b
Taurine (Tau)	21.30a	22.07a
Lysine (Lys)	11.96a	11.48a
Threonine (Thr)	67.35a	68.70a
Glutamic acid (Glu)	42.66a	37.87ab
Alanine (Ala)	11.94b	71.70b
Valine (Val)	71.70b	37.20b
Isoleucine (Ile)	37.20b	42.95b
Leucine (Leu)	42.95b	17.78b
Tyrosine (Tyr)	17.78b	9.01b
Phenylalanine (Phe)	9.01b	53.24b
Aspartic acid (Asp)	53.24b	32.46b
Histidine (His)	32.46b	-
Arginine (Arg)	-	-
Proline (Pro)	-	-

2.3 Effects of Methionine Deficiency on Expression of Methionine Metabolism-Related Genes in Laying Hens' Liver

As shown in Table 5 , dietary methionine level significantly affected the expression levels of MAT1a, Dnmt1, METTL3, METTL14, CBS, and MTR in the liver of laying hens ($P<0.05$), but had no significant effect on the expression levels of Dnmt1, Ahcy, and BHMT ($P>0.05$). The expression levels of Dnmt1, METTL3, and METTL14 in the liver of hens in the 0.21% methionine group were significantly higher than those in the other two groups ($P<0.05$). The METTL3 expression level in the liver of hens in the 0.27% methionine group was significantly higher than that in the control group ($P<0.05$). The expression levels of MAT1a and MTR in the liver of hens in the 0.21% methionine group were significantly higher than those in the other two groups ($P<0.05$), and the CBS expression level was significantly higher than that in the control group ($P<0.05$). The MTR expression level in the liver of hens in the 0.27% methionine group was significantly higher than that in the control group ($P<0.05$).

Table 5 Effects of methionine deficiency on expression of methionine metabolism-related genes in laying hens' liver

Genes	Dietary methionine level/%	P-value
	0.21%	0.27%
Methionine adenosyl-transferase 1a (MAT1a)	1.49a	1.09b
DNA methyl-transferase 1 (Dnmt1)	1.61a	0.96b
DNA methyl-transferase 3a (Dnmt3a)	2.01a	1.29b
m6A methyl-transferase 3 (METTL3)	2.12a	1.16b
m6A methyl-transferase 14 (METTL14)	1.36a	1.17ab
Adenosylhomocysteinase (Ahcy)	1.87a	1.29b
Cystathionine- -synthase (CBS)	1.49a	1.09b
5-methyltetrahydrofolate-homocysteine methyl-transferase (MTR)	1.61a	0.96b
Betaine-homocysteine methyl-transferase (BHMT)	2.01a	1.29b

3.1 Effects of Methionine Deficiency on Production Performance of Laying Hens

Methionine is the first limiting amino acid for laying hens and plays an important role in their production performance. Studies have shown that when methionine content is low, increasing methionine levels can improve laying hen performance, but when dietary methionine reaches a certain level, continued supplementation 反而 reduces performance. In this experiment, dietary methionine levels were all relatively low, and the 0.33% methionine group achieved the highest production performance indicators, though the feed-to-egg ratio was not significantly different from the 0.27% methionine group. Saki et al. reported that increasing methionine level 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 level (0.20%-0.34%) significantly affected laying rate, average egg weight, and average daily feed intake, with no significant effects when methionine level exceeded 0.28%. However, Keshavarz reported that reducing dietary methionine level 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 utilization, but had no significant effect on average daily egg production and average daily feed intake.

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

Serum free amino acid concentrations can reflect amino acid metabolism status in animals to some extent. Due to synergistic, alternative, conversion, and antagonistic relationships among amino acids, deficiency or excess of a particular amino acid in the diet can cause amino acid imbalance and affect the utilization of itself or other amino acids. This study showed that dietary methionine level significantly affected the content and proportion of some amino acids in serum, such as decreasing methionine, cysteine, isoleucine, and arginine contents while increasing serine and glycine contents. Similar to this study, Lü et al. also reported that dietary methionine level affected serine, methionine, and isoleucine contents. Yodseranee et al. also found in broilers that dietary methionine level affected plasma methionine, cysteine, and taurine contents.

3.3 Effects of Dietary Methionine Deficiency on Methionine Metabolism Process in Laying Hens' Liver

Approximately 20% of ingested methionine is digested and metabolized in the gastrointestinal tract, while 80% is transported via blood to cells and tissues, with more than half being converted to S-adenosylmethionine in the liver to enter the methionine metabolic pathway. Methionine is converted to S-adenosylmethionine by methionine adenosyltransferase in an ATP-consuming process, an enzyme primarily encoded by MAT1a. Over 90% of S-adenosylmethionine can subsequently be converted to S-adenosylhomocysteine

by methyltransferases, with the methyl group removed participating in DNA and RNA methylation processes under the action of methyltransferases, thereby regulating gene expression and RNA-mediated cellular pathways. In this experiment, methionine deficiency significantly affected the expression levels of Dnmt1, METTL3, and METTL14, indicating that methionine deficiency may affect DNA and RNA methylation processes. Mattocks et al. found that short-term methionine restriction could improve the efficiency of the DNA methylation maintenance system and increase overall hepatic DNA methylation levels in adult C57BL/6J mice. However, Liu et al. reported that the methylation level of the GC-rich myostatin gene exon 1 region was 46% and 83% in low- and high-methionine groups, respectively, and that if the GC-rich region was already highly methylated, excessive or insufficient dietary methionine intake could cause demethylation. RNA methylation is the most common and abundant modification of RNA, with m6A formed on carbon or nitrogen atoms using methyl groups provided by S-adenosylmethionine. METTL3 is the active component of the m6A methyltransferase complex in mammalian cells, and knockout of the METTL3 gene may cause a 30% decrease in total m6A levels in HeLa cells and apoptosis in HepG2 cells through a p53-mediated pathway. METTL14 is another active component of the m6A methyltransferase complex that can bind to METTL3 protein in a 1:1 stoichiometric ratio to form a stable dimeric complex, regulating the deposition of m6A on mRNA. Currently, there are no reports on the effects of methionine deficiency on RNA methylation. In this experiment, methionine deficiency significantly increased the expression levels of METTL3 and METTL14, suggesting that methionine deficiency may affect RNA methylation levels in a dose-dependent manner.

S-adenosylhomocysteine is decomposed into adenosine and homocysteine by Ahcy in a reversible, non-rate-limiting step, with metabolic flux determined by the synthesis rate of S-adenosylhomocysteine and the consumption rates of adenosine and homocysteine. In this experiment, there were no significant differences in Ahcy expression among the three groups, suggesting that methionine deficiency may not affect the ratio of these metabolites. Homocysteine has two subsequent metabolic pathways: one is remethylation to methionine using 5-methyltetrahydrofolate by MTR and betaine by BHMT as methyl donors, constituting the methionine cycle; the other is transsulfuration to cystathionine catalyzed by CBS with serine participation, which then participates in cysteine, glutathione, or taurine synthesis and oxidative degradation. Demethylated tetrahydrofolate can be regenerated to 5-methyltetrahydrofolate through the cycle, simultaneously converting serine to glycine. In this experiment, methionine deficiency significantly affected the expression levels of MAT1a, CBS, and MTR, indicating that methionine deficiency may affect homocysteine metabolic pathways. Meanwhile, the decreased serum cysteine content and significant accumulation of serine and glycine in the high-dose methionine deficiency group suggest that methionine deficiency may reduce the transsulfuration pathway of methionine while enhancing the remethylation pathway, with the reduction in transsulfuration possibly exceeding the increase in remethylation. Studies have

shown that when methionine is deficient, homocysteine remethylation to methionine can ensure normal methylation reactions, albeit at the cost of reduced -ketobutyrate and glutathione synthesis. Conversely, increasing methionine level by only 10% can double the efficiency of homocysteine synthesis. Methionine loading experiments have shown that methionine loading can activate the transsulfuration pathway of homocysteine in rat hepatocytes while inhibiting its remethylation pathway, significantly reducing BHMT and methionine synthase activities without affecting CBS activity. However, in this experiment, methionine deficiency also significantly increased CBS expression. Tang et al. reported that methionine deprivation could induce tissues to downregulate CBS protein through an S-adenosylmethionine-independent mechanism to efficiently conserve methionine, but at the mRNA level, CBS expression did not decrease and even increased, which is consistent with the results of this study.

Conclusions

1. Methionine deficiency significantly reduced laying rate, average daily egg production, and average egg weight, and increased the feed-to-egg ratio in Hy-Line Grey laying hens during the late laying period.
2. Methionine deficiency significantly increased the expression levels of Dnmt1, METTL3, and METTL14 in the liver, affecting DNA and RNA methylation processes.
3. Methionine deficiency significantly decreased serum methionine content, increased serum glycine and serine contents, and enhanced liver MTR expression, affecting 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-

[4] KIKUSATO M, SUDO S, TOYOMIZU M. Methionine deficiency leads to hepatic fat accretion via impairment of fatty acid import by carnitine palmitoyltransferase [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 affect 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 -synthase[J]. Nutrition, 2010, 26(11/12): 1170-1175.

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