Effects of Excess Methionine on Growth Performance and Blood Indices in 7- to 28-Day-Old Pekin Ducks (Postprint)

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Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of excess dietary methionine on growth performance and blood indices of Peking ducks aged 7–28 days, and to evaluate the maximum safe limit of methionine in diets for Peking ducks aged 7–28 days. A total of 252 healthy male Peking ducks at 7 days of age were selected and randomly divided into 6 groups with 7 replicates per group and 6 ducks per replicate, based on the principle of similar body weight. Each group was fed diets with methionine levels of 0.48%, 0.73%, 0.98%, 1.23%, 1.48%, and 1.73%, respectively. Among these, the normal diet with 0.48% methionine met the normal growth requirements of Peking ducks during the brooding period. The experimental period lasted 21 days. The results showed: 1) Compared with the dietary methionine level of 0.48%, when the dietary methionine level was 0.98% and above, the final weight, average daily feed intake, and average daily gain of the experimental ducks were significantly decreased ($P<0.05$), and further significantly decreased with increasing methionine levels ($P<0.05$). There was no significant difference in feed-to-gain ratio among groups ($P>0.05$). 2) Compared with the dietary methionine level of 0.48%, when the dietary methionine level was 0.98% and above, plasma total bilirubin content was significantly increased ($P<0.05$); when the dietary methionine level was 1.23% and above, plasma homocysteine content was significantly increased ($P<0.05$), and further significantly increased with increasing methionine levels ($P<0.05$); when the dietary methionine level was 1.73%, plasma alanine aminotransferase and lactate dehydrogenase activities were significantly increased ($P<0.05$). 3) Compared with the dietary methionine level of 0.48%, when the dietary methionine level was 0.98% and above, blood hemoglobin content and mean corpuscular hemoglobin concentration were both significantly decreased ($P<0.05$); when the dietary methionine level was 1.23% and above, blood mean corpuscular hemoglobin content was significantly decreased ($P<0.05$); when the dietary methionine level was 1.48%

and above, blood red cell distribution width and mean corpuscular volume were significantly increased (P<0.05), and blood red blood cell count was significantly decreased (P<0.05). 4) Using average daily gain as the evaluation index, the broken-line model estimated that the maximum safe limit of dietary methionine for Peking ducks aged 7–28 days was 0.87%. It can be concluded that dietary excess methionine can significantly inhibit the growth of Peking ducks aged 7–28 days and cause abnormal blood indices. The maximum safe limit of dietary methionine for Peking ducks aged 7–28 days is 0.87%.

Full Text

Effects of Excess Methionine on Growth Performance and Blood Parameters of Peking Ducks during 7 to 28 Days of Age

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Abstract

This experiment was conducted to investigate the effects of dietary excess methionine on growth performance and blood parameters of Peking ducks during 7 to 28 days of age and to evaluate the maximum safe level of methionine in diets for this age group. A total of 252 seven-day-old healthy male Peking ducks were randomly allocated to six groups with seven replicates per group and six ducks per replicate based on similar initial body weight. The six groups were fed diets containing methionine levels of 0.48%, 0.73%, 0.98%, 1.23%, 1.48%, and 1.73%, respectively. The diet with 0.48% methionine was considered normal and met the normal growth requirements for starter Peking ducks. The experimental period lasted 21 days. The results showed that: 1) Compared with the 0.48% methionine diet, when dietary methionine level reached 0.98% or above, final body weight, average daily feed intake, and average daily gain of the experimental ducks were significantly reduced (P<0.05), with further significant decreases as methionine level increased (P<0.05). No significant differences were observed in feed-to-gain ratio among groups (P>0.05). 2) Compared with the 0.48% methionine diet, plasma total bilirubin content was significantly elevated when dietary methionine level reached 0.98% or above (P<0.05); plasma homocysteine content was significantly increased at methionine levels of 1.23% or above (P<0.05), with further significant increases as methionine level rose (P<0.05); and plasma alanine aminotransferase and lactate dehydrogenase activities were significantly elevated at 1.73% methionine (P<0.05). 3) Compared

with the 0.48% methionine diet, blood hemoglobin content and mean corpuscular hemoglobin concentration were significantly decreased at methionine levels of 0.98% or above ($P<0.05$); mean corpuscular hemoglobin content was significantly reduced at 1.23% methionine or above ($P<0.05$); and red cell distribution width and mean corpuscular volume were significantly increased while red blood cell count was significantly decreased at methionine levels of 1.48% or above ($P<0.05$). 4) Using average daily gain as the evaluation index, broken-line model analysis estimated the maximum safe level of methionine in diets for 7 to 28-day-old Peking ducks to be 0.87%. In conclusion, dietary excess methionine significantly inhibited growth and caused abnormal blood parameters in 7 to 28-day-old Peking ducks, with a maximum safe dietary methionine level of 0.87%.

Keywords: methionine; ducks; growth performance; safe level; blood parameters

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Methionine is an essential amino acid for meat duck growth and development, participating in important physiological and biochemical processes including protein synthesis, methyl metabolism, and biological antioxidant activity. Since meat duck compound feeds are primarily composed of plant-based ingredients such as corn, soybean meal, and miscellaneous meals that are low in methionine, methionine is the first limiting amino acid in meat duck diets. Consequently, crystalline methionine must be added to meat duck diets to meet growth requirements. Appropriate dietary methionine levels can promote duck growth, improve carcass quality, and enhance feather development. However, methionine is also a highly toxic amino acid. Adding 1% crystalline methionine to a diet with normal methionine level (0.38%) significantly reduced average daily gain and average daily feed intake in 21 to 42-day-old Peking ducks. Currently, no studies have reported on the safe limit of methionine in meat duck diets, and published studies on methionine toxicity are limited by few dietary methionine levels, making accurate prediction of safe limits difficult. The broken-line model has been successfully applied to predict maximum safe levels of feed ingredients in poultry, providing a new statistical approach for studying methionine safety limits. Since naturally occurring methionine is primarily the L-isomer, this experiment used crystalline L-methionine as the methionine source, adding different levels to a basal diet that already met nutritional requirements, to investigate the effects of excess methionine on growth performance and blood parameters of 7 to 28-day-old Peking ducks and to predict the maximum safe level using a broken-line model, thereby providing technical parameters and theoretical guidance for the rational and safe use of crystalline methionine in meat duck diets.

1.1 Experimental Design and Diet Composition

A single-factor completely randomized design was employed with six dietary methionine levels (0.48%, 0.73%, 0.98%, 1.23%, 1.48%, and 1.73%). The basal diet was formulated according to China' s "Feeding Standard of Meat Ducks" (NY/T 2122-2012), with a methionine level of 0.48% that met the normal growth requirements for starter Peking ducks. The composition and nutrient levels of the basal diet are shown in Table 1 . Six experimental diets with different methionine levels were prepared by adding crystalline L-methionine (99% purity) at six supplementation levels (0, 0.25%, 0.50%, 0.75%, 1.00%, and 1.25%) to the basal diet. All experimental diets were pelleted.

Based on the principle of similar initial body weight per replicate, 252 seven-day-old healthy male Peking ducks were randomly divided into six groups with seven replicates per group and six ducks per replicate. The experimental period lasted 21 days.

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

Items	Content
Ingredients	
Corn	
Soybean meal	
NaCl	
CaHPO	
Limestone	
Premix[1]	
L-Met	
Soybean oil	
Total	
Nutrient levels[2]	
ME/(MJ/kg)	
CP	
AP	
Met	
Met+Cys	
Lys	
Trp	
Arg	
Thr	

[1]The premix provided the following per kilogram of diet: Cu 10 mg, Fe 60 mg, Zn 60 mg, Mn 80 mg, Se 0.3 mg, I 0.2 mg, choline chloride 1,000 mg, VA 10,000 IU, VD 3,000 IU, VE 20 IU, VK 2 mg, thiamin 2 mg, riboflavin 8 mg, VB 4 mg, VB 0.02 mg, pantothenic acid 20 mg, folic acid 1 mg, biotin 0.2 mg.
[2]Nutrient levels were all calculated values.

1.2 Management

Ducks were raised on wire floors with ad libitum access to feed and water under 24-hour lighting. From 7 to 28 days of age, ducks were fed according to experimental diet groups, while other management practices followed conventional procedures.

1.3 Growth Performance Measurement

On the morning of day 29, fasting body weight and remaining feed weight were measured for each replicate to calculate average daily gain, average daily feed intake, and feed-to-gain ratio (F/G) for days 7 to 28.

1.4 Blood Parameter Measurement

On the morning of day 29, two ducks were randomly selected from each replicate per group, and 6 mL of blood was collected via jugular vein puncture. Three milliliters were placed in Na EDTA anticoagulant tubes for routine blood parameter analysis, while the remaining 3 mL were placed in heparin sodium anticoagulant tubes, centrifuged at 3,500 r/min for 15 minutes to separate plasma for plasma biochemical parameter analysis.

Blood routine parameters including hemoglobin (Hb) content, red blood cell count (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and red cell distribution width (RDW) were analyzed using a German ABX Pentra 120 hematology analyzer with reagent kits purchased from ABX Company.

Plasma total protein (TP), albumin (ALB), uric acid (UA), total bilirubin (TBIL) content, and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) activities were analyzed using a HITACHI 7080 automatic biochemical analyzer with reagent kits purchased from Sichuan Mike Biological Technology Co., Ltd. Plasma homocysteine (Hcy) content was analyzed by pre-column fluorescence derivatization high-performance liquid chromatography according to the method of Xie [11] using a Waters 2690 liquid chromatograph.

1.5 Statistical Analysis

Experimental data were expressed as "mean ± standard deviation" and analyzed using SAS 9.3 statistical software. The GLM procedure was applied for one-way ANOVA according to a completely randomized design, with $P<0.05$ as the significance level. Duncan' s multiple range test was used for mean comparisons. Following the method of Alhotan et al. [10], a broken-line model was used to predict the maximum safe level of methionine in meat duck diets.

The broken-line model was as follows:

$$\mathbf{y = l + u(x - r)}$$

where y represents duck production performance, x represents dietary methionine level (%), r represents the maximum safe level of methionine (%), l represents duck production performance when $x = r$, and u represents the slope of the broken-line model. In this model, when $x < r$, $y = l$.

2.1 Effects of Excess Methionine on Growth Performance of 7 to 28-Day-Old Peking Ducks

As shown in Table 2 , excess methionine significantly affected final body weight, average daily gain, and average daily feed intake of Peking ducks (P<0.05). Compared with dietary methionine levels of 0.48% and 0.73%, the 0.98% methionine diet significantly reduced final body weight (P<0.05) and significantly decreased average daily gain and average daily feed intake (P<0.05), with further significant reductions as dietary methionine level increased (P<0.05). No significant differences were observed in feed-to-gain ratio among groups (P>0.05).

Table 2 Effects of excess methionine on growth performance of Peking ducks during 7 to 28 days of age

Items	Dietary methionine level/%	P-value
Initial body weight/g	145.4±1.9	144.4±2.1
Final body weight/g	1,820.6±34.3[a]	1,828.0±53.5[a]
ADFI/g	151.0±2.4[a]	149.2±5.4[a]
ADG/g	79.8±1.6[a]	80.2±2.5[a]
F/G	1.89±0.02	1.86±0.04

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

2.2 Effects of Excess Methionine on Plasma Biochemical Parameters of 28-Day-Old Peking Ducks

As shown in Table 3 , excess methionine significantly affected plasma total bilirubin and homocysteine contents and alanine aminotransferase and lactate dehydrogenase activities (P<0.05), but had no significant effects on total protein, albumin, uric acid contents, or aspartate aminotransferase activity (P>0.05). Compared with the 0.48% methionine diet, plasma total bilirubin content was significantly elevated at dietary methionine levels of 0.98% or above (P<0.05); plasma homocysteine content was significantly increased at methionine levels of 1.23% or above (P<0.05), with further significant increases as methionine level rose (P<0.05); and plasma alanine aminotransferase and lactate dehydrogenase activities were significantly elevated at 1.73% methionine (P<0.05).

Table 3 Effects of excess methionine on plasma biochemical parameters of Peking ducks at 28 days of age

Items	Dietary methionine level/%	P-value
TP/(g/L)	12.8±2.0	13.2±2.4
ALB/(g/L)	15.3±0.8	15.0±1.0
UA/(mol/L)	147.5±27.7	141.7±32.4
TBIL/%	6.7±0.9	7.5±0.4
AST/(U/L)	10.6±1.9	9.3±1.2
ALT/(U/L)	30.0±4.7	29.9±4.8
LDH/(U/L)	274.8±34.6	281.1±71.3
Hcy/(mol/L)	16.0±3.2	15.0±1.5

2.3 Effects of Excess Methionine on Routine Blood Parameters of 28-Day-Old Peking Ducks

As shown in Table 4 , excess methionine significantly affected blood hemoglobin content, red blood cell count, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin content, mean corpuscular volume, and red cell distribution width ($P<0.05$). Compared with the 0.48% methionine diet, blood hemoglobin content and mean corpuscular hemoglobin concentration were significantly decreased at methionine levels of 0.98% or above ($P<0.05$); mean corpuscular hemoglobin content was significantly reduced at 1.23% methionine or above ($P<0.05$); and red cell distribution width and mean corpuscular volume were significantly increased while red blood cell count was significantly decreased at methionine levels of 1.48% or above ($P<0.05$).

Table 4 Effects of excess methionine on routine blood parameters of Peking ducks at 28 days of age

Items	Dietary methionine level/%	P-value
Hb/(g/L)	107.9±3.1[a]	102.79±7.7
RBC/($\times 10^{12}$/L)	2.3±0.1[a]	2.2±0.1
MCHC/(g/L)	280.6±6.6[a]	277.2±6.4[a]
MCH/(pg)	47.1±1.0[a]	46.3±1.0
MCV/fL	166.3±2.6	167.5±2.5
RDW/%	7.3±0.2	7.3±0.2

Maximum Safe Level of Methionine in Diets for 7 to 28-Day-Old Peking Ducks

Using broken-line model regression analysis, the regression equation between dietary methionine level (x) and average daily gain (y) was obtained: $\mathbf{y = 79.97 - 33.41 \times (x - 0.87)}$ (Figure 1 [Figure 1: see original paper]). According to this regression equation, the maximum safe level of methionine in diets for 7 to 28-day-old Peking ducks was 0.87%.

Figure 1 Regression relationship between dietary methionine level and average daily gain of Peking ducks during 7 to 28 days of age

3.1 Effects of Excess Methionine on Growth Performance of 7 to 28-Day-Old Peking Ducks

Compared with other amino acids, methionine has a relatively narrow tolerance range and obvious toxic effects, attracting considerable research attention. Chen et al. [13] reported that adding 1.02% DL-methionine to a semi-purified diet significantly reduced body weight and feed conversion efficiency in 21-day-old broilers while significantly increasing the incidence of tibial chondrodysplasia. Han et al. [14] found that adding 1.00%, 2.00%, and 4.00% DL-methionine to a corn-soybean meal basal diet reduced average daily gain, average daily feed intake, and feed conversion efficiency in 8 to 22-day-old broilers. Baker et al. [15] demonstrated that adding 1.75% L-methionine to a purified basal diet (0.35% methionine + 0.35% cysteine) significantly reduced average daily gain and feed-to-gain ratio in broiler chicks. Xie et al. [9] observed that both DL-methionine (1.00% and 2.00%) and equimolar concentrations of DL-hydroxy analogue free acid significantly inhibited average daily gain and average daily feed intake in growing Peking ducks. In the present study, average daily gain and average daily feed intake of Peking ducks were significantly reduced when dietary methionine level exceeded 0.98%, consistent with the aforementioned reports. The significant reduction in average daily feed intake with increasing dietary methionine levels may be due to competitive inhibition of amino acid uptake sharing the same transporters, leading to depletion of these amino acids in the animal brain and subsequent central nervous system feedback inhibition of feed intake of amino acid-imbalanced diets. Some studies have suggested this may be related to elevated insulin levels, though the specific mechanism requires further investigation. In this experiment, the significant decrease in average daily feed intake was accompanied by a significant decline in average daily gain, while feed-to-gain ratio remained unchanged, indicating that the inhibitory effect of excess methionine on feed intake was the primary reason for restricted growth in meat ducks.

3.2 Effects of Excess Methionine on Plasma Biochemical Parameters of 28-Day-Old Peking Ducks

Blood biochemical parameters are important indicators reflecting metabolic status and can, to some extent, reflect animal growth and health status, with wide applications in animal production, nutritional regulation, and disease diagnosis. Homocysteine is an intermediate metabolite of methionine metabolism, and plasma homocysteine is a sensitive indicator of methionine toxicity. In this study, plasma homocysteine content was significantly elevated when dietary methionine level reached 1.23% or above, reflecting not only methionine toxicity in meat ducks but also enhanced methionine catabolism leading to accumulation of homocysteine as an intermediate metabolite. Current medical

research indicates that excessively high plasma homocysteine is an independent risk factor for cardiovascular disease in humans, with the occurrence of cardiovascular and cerebrovascular diseases closely related to vascular endothelial cell damage caused by elevated homocysteine. Plasma homocysteine levels in patients with cardiovascular diseases such as coronary heart disease, stroke, and acute cerebrovascular disease are significantly higher than in healthy individuals. Therefore, excessively high homocysteine levels may be one of the mechanisms underlying methionine toxicity in meat ducks. Additionally, alanine aminotransferase and aspartate aminotransferase are primarily located in hepatocytes and are normally present in low concentrations in blood, but are massively released into circulation when liver cells are damaged, resulting in significantly elevated blood transaminase activities. Thus, blood transaminase activity is a sensitive indicator of liver injury. Plasma total bilirubin content, the sum of direct and indirect bilirubin, can reflect abnormalities in liver secretion and excretion functions when elevated. In this study, excess dietary methionine caused significant increases in plasma total bilirubin content and alanine aminotransferase activity. Han et al. [17] also found that excess methionine significantly increased plasma alanine aminotransferase activity while inhibiting growth in 3 to 6-week-old Peking ducks, consistent with our results and further suggesting that dietary methionine can cause liver injury in meat ducks.

3.3 Effects of Excess Methionine on Routine Blood Parameters of 28-Day-Old Peking Ducks

Blood consists of plasma and blood cells, and routine blood examination primarily involves quantitative detection of blood cells, which can effectively reflect health status. Currently, few studies have investigated the effects of dietary methionine levels on animal blood routine parameters. In human medicine, hemoglobin content, red blood cell count, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin content, mean corpuscular volume, and red cell distribution width are primarily used for anemia diagnosis and inferring disease etiology. Mean corpuscular volume and red cell distribution width have good clinical value for anemia classification, and when both are elevated, "megaloblastic anemia" is often diagnosed. Megaloblastic anemia is a type of nutritional anemia mainly caused by folic acid and vitamin B deficiency, accompanied by elevated homocysteine levels. In this study, excessive dietary methionine caused significant reductions in blood hemoglobin content, red blood cell count, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin content, along with significant increases in mean corpuscular volume and red cell distribution width. These results not only indicate damage to red blood cells by excess methionine but also suggest that excess methionine may cause megaloblastic anemia in meat ducks, likely related to the significantly elevated plasma homocysteine levels observed in this study. In animals, the remethylation pathway in the methionine cycle is an important route for homocysteine degradation, which requires participation of folic acid and vitamin B . Therefore, intake of excess methionine enhances homocysteine

degradation pathways, thereby increasing the demand for folic acid and vitamin B .

Maximum Safe Level of Methionine in Diets for 7 to 28-Day-Old Peking Ducks

Studies have shown that appropriate dietary methionine supplementation can significantly improve animal growth performance and product quality, but excess methionine inhibits normal growth and can cause death under severe conditions. The present study also demonstrated obvious toxic effects of excess dietary methionine on 7 to 28-day-old Peking ducks. Therefore, determining the maximum safe level of methionine is important for practical meat duck production. Recently, Alhotan et al. [10] evaluated the effectiveness of the broken-line model in predicting maximum safe levels and found that it better predicted maximum safe levels of feed ingredients. In this study, using average daily gain as the evaluation index, the broken-line model estimated the maximum safe level of methionine in diets for 7 to 28-day-old Peking ducks to be 0.87%. When dietary methionine levels were 0.57%, 0.46%, and 0.51% in starter broilers, adding 1.00% methionine did not significantly inhibit average daily gain. However, the estimated maximum safe level in this study was lower than 1%, indicating that excess methionine has a stronger growth-inhibiting effect on starter Peking ducks than on broiler chicks.

Conclusion

Under the conditions of this experiment, dietary excess methionine inhibited growth and caused abnormal blood parameters in 7 to 28-day-old Peking ducks. Using average daily gain as the evaluation index, the broken-line model estimated the maximum safe level of methionine in diets for this age group to be 0.87%.

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