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Source: Canadian Journal of Animal Science, 103(1) : 73-80

Published By: Canadian Science Publishing

URL: https://doi.org/10.1139/cjas-2021-0101

Canadian Journal of Animal Science

Article

Response of growth performance, blood hematology, organ indexes, and myofiber traits to increasing dietary methionine levels in Jilin White goose

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Abstract

A total of 240 geese (28 days old; 120 ganders and 120 gooses) with an average initial body weight of 1068.19 ± 6.59 g were used to evaluate the effects of increasing dietary methionine (Met) levels on growth performance, blood hematology, organ indexes, and myofiber traits. The experimental period was 42 days. All birds were randomly assigned to four treatment groups based on the initial body weight. There were six replicate cages per treatment, and 10 geese per cage (5 ganders and 5 gooses). Dietary treatments were based on a basal diet containing 0.25% Met, and extra supplied 0.25%, 0.50%, and 0.75% Met to form different dietary groups (0.25%, 0.50%, 0.75%, and 1.00% Met, as-fed basis). The results of this study indicated that final body weight, body weight gain, and feed efficiency increased quadratically, relative weight of breast muscle and myofiber diameter increased cubically, serum total protein and uric acid concentrations, relative weight of liver and abdominal fat, and myofiber diameter increased linearly, whereas myofiber density decreased linearly, with the level of Met increased. The maximized growth performance and breast muscle parameters were observed in 0.75% Met-containing group.

Key words: goose, growth performance, myofiber traits, methionine, organ index

Introduction

Methionine (Met) is the first limiting amino acid for poultry (Rehman et al. 2019; Lee et al. 2021). Met is a key nutrient ingredient in the diet for maintaining healthy growth and general production (Bunchasak 2009). Met deficiency in diet will decline breast muscle accretion (Wen et al. 2014), depress growth (Wu et al. 2021), impair immune status (Wu et al. 2012), reduce protein synthesis (Lee et al. 2021), decrease carcass edible components (Wu et al. 2019), and lead to metabolic disorders (Kikusato et al. 2015). Therefore, maintaining the normal growth and production of poultry requires sufficient Met in the diet.

In the past, Nitsan et al. (1983) evaluated the Met requirement of geese, and noted that hatched to 2-week-old geese required 0.29% dietary Met level, while geese aged 2–7 weeks required 0.15% dietary Met level. Leclerq et al. (1987) reported that the requirement of Met for 4–6-week-old geese was 0.29%–0.31%, while that for 7–12-week-old geese was 0.25%–0.27%. Modern goose breeding company recommended that the Met requirement in growing gosling was 0.30%, while that of breeder gosling was 0.25% (https://www.dpi.nsw.gov.au/). However, NRC (1994) does not provide a clear recommendation for the Met requirement of geese. Therefore, it is necessary to evaluate the response of growth and productive performance of geese to different dietary Met levels.

Feeding growing goslings with 0.28% Met-containing basal diet and supplemented with 0.06% and 0.12% Met (0.28%, 0.34%, and 0.40% Met, as-fed basis) has been reported to significantly improve body weight and body weight gain (BWG) (Yang et al. 2016, 2017). Increasing dietary Met levels (0.23%, 0.33%, 0.43%, 0.43%, and 0.63% Met, as-fed basis) in geese could increase BWG and gain-to-feed ratio (G:F) and decrease relative weight of abdominal fat (Wang et al. 2010). In addition, Yang et al. (2018) noted that the body weight and BWG in geese fed with 0.40% Met-containing diet were higher than those of geese fed with 0.28% Met-containing diet.

However, more studies are needed to evaluate the effects of dietary Met supplementation on growth and productive performance in geese. In addition, the breed of geese used in the above studies is Yangzhou geese, which is different from this study. Evaluating the response of growth and productive performance in different breeds of geese to the increase of dietary Met levels is helpful to determine the Met requirement of geese. In the present study, dietary Met allowance was set as 0.25%, 0.50%, 0.75%, and 1.00%. In addition, the breed of geese was Jilin White goose. We hypothesized that increasing dietary Met levels could improve growth performance, organ

indexes, and myofiber traits in geese. The objective of this study was to evaluate the response of growth performance, blood hematology, organ indexes, and myofiber traits to the variation of dietary Met levels (0.25%, 0.50%, 0.75%, and 1.00% Met, as-fed basis) in Jilin White goose.

Materials and methods

All procedures of this study were approved by the Institutional Animal Care and Use Committee of Jilin Agricultural University (Changchun, China). Animals were handled according to the guidelines described by the Canadian Council on Animal Care (2009).

Animals and housing

A total of 240 28-day-old Jilin White geese (120 ganders and 120 gooses) with an average initial body weight of 1068.19 ± 6.59 g were used in a randomized complete block design experiment. All geese were housed in a temperature-controlled room with continuous lighting. The temperature of the room was maintained at 24 °C and then reduced by 2 °C per week to a final temperature of 20 °C.

Experimental design and diets

Based on the initial body weight, geese were randomly assigned to four treatments, with six replicate cages and 10 geese per replicate (5 ganders and 5 gooses). The experimental period was 42 days (28–70 days of age). All birds had free access to feed and water throughout the experimental period. Dietary treatments were based on a corn–soybean meal basal diet containing 0.25% Met, and extra supply with 0.25%, 0.50%, and 0.75% Met to form four dietary groups (0.25%, 0.50%, 0.75%, and 1.00% Met, as-fed basis). The basal diet was formulated based on NRC (1994) (Table 1).

Table 1. Composition and nutrient levels of the experimental basal diet (%, as-fed basis).

Ingredients, %	
Corn	50.87
Soybean meal	24.94
Wheat bran	5.10
Fish meal	1.70
Lysine-HCl	0.17
Lucerne	10.00
Maize stalk	5.00
Dicalcium phosphate	0.72
Limestone	0.70
Sodium chloride	0.30
Vitamin and trace mineral premix[1]	0.50
Total	100.00
Calculated value, %	
Metabolizable energy, MJ/kg	10.40
Calcium	0.80
Available phosphorus	0.36
Hemicelluloses	0.16
Analyzed composition, %	
Crude protein	18.89
Methionine	0.25
Total sulfate amino acid	0.48
Lysine	0.95
Crude fiber	0.66
Neutral detergent fiber	0.28
Acid detergent fiber	0.12

[1]Provided per kg of complete diet: Cu, 10 mg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg; Se, 0.3 mg; I, 0.2 mg; Cr, 0.15 mg; choline chloride, 1000 mg; vitamin A (retinyl acetate), 10 000 IU; vitamin D_3, 2000 IU; vitamin E (DL-α-tocopheryl acetate), 10 IU; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 8 mg; pyridoxine, 2 mg; vitamin B_{12}, 0.02 mg; pantothenicacid, 20 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.2 mg.

Sample collection and measurements

Feed analysis

After homogeneous mixing, feed samples were collected from each dietary group. All feed samples were dried in a 70 °C constant temperature oven for 72 h. Subsequently, feed samples were ground and sieved with a 1 mm sieve. Feed powder with a diameter of less than 1 mm was collected for feed composition analysis. According to the procedure established by AOAC (2000), the dry matter (method 930.15), crude protein (nitrogen × 6.25; method 968.06), and crude fiber (method 991.43) composition in the diet was analyzed. Then, the representative feed samples in each group were hydrolyzed with 6 *N* HCl for 24 h at 110 °C. An amino acid analyzer (2690 Alliance, Waters, Inc., Milford, MA, USA) was used for determining amino acid contents in the diet. In addition, the contents of neutral detergent fiber and acid detergent fiber in the diet were measured according to the method provided by Mertens (2002).

Growth performance

All geese were weighed on days 1, 21, and 42 after feed deprivation for 12 h for measuring body weight gain (BWG). Cage-based feed intake was checked daily to measure average daily feed intake (ADFI). The G:F was calculated using BWG and ADFI values.

Blood hematology analysis

At the end of the experiment, two geese (one gander and one goose) per cage were selected randomly and blood samples were collected via wing vein and immediately centrifuged (3000*g*) for 15 min at 4 °C to obtain serum samples. The concentrations of total protein, uric acid, albumin, malondialdehyde (MDA), and superoxide dismutase (SOD) were measured with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) using an Olympus AU640 analyzer (Olympus, Tokyo, Japan).

Relative weight of organ analysis

After blood collection, the geese were slaughtered by cervical dislocation. The breast muscle, liver, abdominal fat, bursa of fabricius, spleen, and thymus were removed and weighed to calculate the organ indexes. The organ indexes were calculated using the following equation:

$$\text{Organ index (\%)} = \frac{\text{Organ weight}}{\text{Live body weight}} \times 100$$

Myofiber trait analysis

After slaughtering the geese, the breast muscle samples were cut into small pieces and fixed with 10% neutral buffered formalin for 12 h, followed by dehydration in increasing concentrations of alcohol (70%, 80%, 90%, 95%, and 100%) and xylene. Consequently, samples were embedded in paraffin and stored in an oven at 60 °C. Twelve hours later, samples were removed from the oven and histological cassettes. Fragments were placed in "paper boxes" and covered with paraffin. After the paraffin solidified into blocks, the "papers" were removed and the blocks were kept under refrigeration until the cuts were realized (Felício et al. 2013).

Serial tissue sections (3 μm thickness) were excised perpendicular to the direction of the myofibers using a cryostat. After sectioning, the paraffin section ribbon was put on the coating slide glass. Dried slides were kept in oven at 60 °C for 2 h to eliminate any excess paraffin. The next step consisted of paraffin removal and slide hydration, using xylene and different concentrations of ethanol. Samples were then stained following the hematoxylin and eosin staining protocol (Felício et al. 2013).

Samples were then dehydrated again and mounted. In each specimen, the diameters of muscle fibers and muscle fiber density were measured under a light microscope equipped with a ScopePhpto (LY-WN 300, Hangzhou Scopetek Opto-Eletric Co., Ltd.).

No less than 150 intact, well-oriented muscle fibers' cross-sectional area of five fields of vision was measured under 40× objective lens. With muscle fibers assumed to be round, the muscle fiber cross-sectional area (A) was converted to diameter (D) by the following formula: $D = 2\sqrt{A/\pi}$. The average value was calculated to represent the diameter of the muscle fibers (Liu et al. 2019).

The image analysis software was used to measure the total area (S) and the number of muscle fibers (N) of five randomly selected fields of vision. The density (d) was calculated by the following formula: $d = N/S$, and the average value of the five visual fields was taken as the muscle fiber density (Liu et al. 2019).

Statistical analysis

Data were subjected to statistical analysis in a randomized completely block design using the general linear model procedure (SAS Institute Inc., Cary, NC, USA). The replicate cage was used as the experimental unit. The effect of increasing concentration of dietary Met was determined by orthogonal polynomial contrasts. The model included linear, quadratic, and cubic contrasts for effects of supplemental Met. Variability in the data was expressed as the standard error of means (SEM), and $P < 0.05$ was considered statistically significant. The linear and cubic relationships were compared using the REG procedure and TRANSREG procedure of SAS, respectively.

Results

Final body weight (quadratic, $P = 0.036$), BWG (quadratic, $P = 0.037$), and G:F (quadratic, $P = 0.027$) were improved with increasing dietary Met levels. However, extra Met supply did not affect the ADFI. In addition, the highest body weight, BWG, and G:F were observed in geese receiving 0.75% Met-containing diet compared with other groups during the experimental period (Table 2).

A linear increase of serum total protein ($P = 0.012$) and uric acid ($P = 0.039$) concentrations was observed with the increase of Met levels in the diet. However, the variation of dietary Met levels did not affect the serum albumin, SOD, and MDA concentrations (Table 3).

Increasing dietary Met levels linearly increased the relative weight of liver ($P = 0.029$) and abdominal fat ($P = 0.025$), while cubically improved the relative weight of breast muscle ($P = 0.045$). Increasing dietary Met levels had no effects on the relative weight of bursa of fabricius, spleen, and thymus of geese (Table 4).

Density of myofiber linearly decreased with increasing dietary Met levels ($P = 0.004$). Moreover, diameter of myofiber linearly ($P < 0.001$) and cubically ($P = 0.009$) increased with increasing dietary Met levels (Table 5).

Discussion

It has been reported that increasing dietary Met levels was beneficial to the growth and productive performance of poultry (Yang et al. 2018). However, excess Met supplementation also induced toxic effects in poultry (Xue et al. 2018). The reduction of feed intake was considered as the characteristic of toxicity response in hyper-Met supplementation (Xie et al. 2007; Faulyi et al. 2015). In the present study, ADFI of geese was not decreased by increasing dietary Met levels. Therefore, the Met levels used in this study could be considered as a tolerable excessive level that would not induce toxic effects on the growth and productive performance of geese.

The growth of poultry is sensitive to the variation of dietary Met levels. It has been reported that birds fed a diet with Met levels higher than the recommendation of NRC could improve the growth performance (Peng et al. 2018; Rehman et al. 2019). Growth is a complex phenomenon; muscle accretion and obesity could induce an increase in body weight (Chen et al. 2006; Wen et al. 2014). Protein synthesis is necessary for muscle accretion (Handique et al. 2019). Concentrations of serum total protein directly reflect the capacity of protein synthesis in vivo (Wu et al. 2021). High protein synthesis capacity in vivo usually corresponds to a high percentage of breast muscle and body weight in poultry (Wen et al. 2014; Ghavi et al. 2020; Sahebi-Ala et al. 2021). In the

Table 2. The response of growth performance to increasing dietary DL-methionine (DL-Met) levels in Jilin White geese.

	Dietary DL-Met levels					*P* value			
Items	0.25%	0.50%	0.75%	1.00%	SEM	ANOVA	Linear	Quadratic	Cubic
Body weight, g									
Day 1	1067.80	1069.63	1079.80	1055.53	6.586	0.110	0.583	0.246	0.385
Day 42	3204.30b	3386.00ab	3567.00a	3355.00ab	49.483	0.065	0.108	0.036	0.295
BWG, g	50.87b	55.15ab	59.22a	54.75ab	1.105	0.058	0.079	0.037	0.318
ADFI, g	236.35	229.76	229.26	230.48	4.010	0.591	0.541	0.555	0.881
G:F	0.22b	0.24ab	0.26a	0.24ab	0.005	0.041	0.052	0.027	0.398

Note: BWG, body weight gain; ADFI, average daily feed intake; SEM, standard error of the mean. Different letters within a row indicate a significant difference ($P < 0.05$).

Table 3. The response of blood hematology to increasing dietary DL-methionine (DL-Met) levels in Jilin White geese.

	Dietary DL-Met levels					*P* value			
Items	0.25%	0.50%	0.75%	1.00%	SEM	ANOVA	Linear	Quadratic	Cubic
Total protein, g/dL	1.24b	1.22b	1.28a	1.29a	0.018	0.025	0.012	0.346	0.104
Uric acid, μmol/L	300.30b	358.70ab	358.00ab	426.00a	38.582	0.043	0.039	0.917	0.539
Albumin, g/L	16.80	16.03	16.20	17.57	0.313	0.197	0.297	0.063	0.907
MDA, nmol/mL	0.04	0.04	0.03	0.04	0.003	0.235	0.428	0.145	0.211
SOD, U/mL	0.17	0.20	0.18	0.18	0.007	0.333	0.881	0.088	0.141

Note: MDA, malondialdehyde; SOD, superoxide dismutase; SEM, standard error of the mean. Different letters within a row indicate a significant difference ($P < 0.05$).

Table 4. The response of organ indexes to increasing dietary DL-methionine (DL-Met) levels in Jilin White geese.

	Dietary DL-Met levels					*P* value			
Items, %	0.25%	0.50%	0.75%	1.00%	SEM	ANOVA	Linear	Quadratic	Cubic
Liver	19.29c	20.83bc	22.34ab	24.37a	0.889	0.005	0.029	0.868	0.934
Breast muscle	55.36b	54.13b	68.15ab	61.00ab	3.845	0.023	0.085	0.449	0.045
Abdominal fat	6.85b	8.32ab	13.96a	12.25ab	2.039	0.028	0.025	0.442	0.218
Bursa of fabricius	0.77	0.71	0.67	0.60	0.154	0.886	0.436	0.966	0.940
Spleen	0.96	0.88	0.99	0.66	0.087	0.389	0.237	0.400	0.345
Thymus	2.29	2.74	2.48	2.23	0.312	0.661	0.744	0.278	0.615

Note: SEM, standard error of the mean. Different letters within a row indicate a significant difference ($P < 0.05$).

Table 5. The response of myofiber traits to increasing dietary DL-methionine (DL-Met) levels in Jilin White geese.

	Dietary DL-Met levels					*P* value			
Items	0.25%	0.50%	0.75%	1.00%	SEM	ANOVA	Linear	Quadratic	Cubic
Density of myofiber, $\times 10^3$ bundle·mm^{-2}	37.04a	25.50ab	11.59b	10.76b	6.919	0.028	0.004	0.443	0.619
Diameter of myofiber, μm	82.08b	81.78b	89.48a	89.38a	1.346	<.001	<.001	0.942	0.009

Note: SEM, standard error of the mean. Different letters within a row indicate a significant difference ($P < 0.05$).

present study, the improvement of body weight, BWG, relative weight of breast muscle, and serum total protein concentration was observed with increasing dietary Met levels. Therefore, we considered that the increase of dietary Met levels could promote the protein synthesis in vivo, which was manifested in the increase of serum total protein concentration, thus increasing the relative weight of breast muscle and being beneficial to the improvement of growth performance, which was consistent with the opinions of Wen et al. (2017) and Sahebi-Ala et al. (2021). In addition, the relative weight of abdominal fat was increased linearly with the level of Met increased in the diet, which probably means the obesity of birds (Chen et al. 2006). However, several studies have reported the converse result; they noted that increasing dietary Met levels would lead to the decrease of abdominal fat deposition in birds (Chattopadhyay et al. 2006; Majdeddin et al. 2019). Effects of increasing dietary Met levels on the relative weight of abdominal fat need to be further discussed. We considered that the increase of abdominal fat percentage was also beneficial to the increase of body weight in geese. In this study, final body weight provided significant fit to quadratic models: $Y = 2727.8 + 2221.4X - 1574.5X^2$, where Y is the body weight

and X is the dietary Met level. In addition, BWG provided significant fit to quadratic models: $Y = 40.132 + 50.036X - 35.002X^2$, where Y is the BWG and X is the dietary Met level. The improvement of G:F was also observed with increasing dietary Met levels. Del Vesco et al. (2013) mentioned that increasing dietary Met levels would decrease the expression of uncoupling protein in the muscle, which results in the energy being used for ATP synthesis instead of heat production (Ledesma et al. 2002; Cannon et al. 2006). This means the improvement of energy utilization in the body. On the other hand, the increase of G:F can also be explained as promoting the synthesis of protein in vivo through increasing dietary Met levels (Angelo 2018). This means the improvement of nitrogen utilization in the body. Therefore, we considered that the improvement of G:F was related to the promotion of protein synthesis in vivo through increasing dietary Met levels, which was manifested in the increase of the relative weight of breast muscle, which was consistent with the opinions of Majdeddin et al. (2019) and Sahebi-Ala et al. (2021). During days 1–42, G:F provided significant fit to the quadratic model: $Y = 0.16 + 0.2607X - 0.1812X^2$, where Y is the feed efficiency and X is the dietary Met level. In brief, increasing dietary Met levels could improve the growth performance of geese through improving breast muscle accretion.

It has been reported that increasing dietary Met levels could lead to an increase in breast muscle percentage (Albrecht et al. 2019; Jiang et al. 2019). It has been proved that Met supplementation could promote muscle anabolism and reduce catabolism, thus favoring protein synthesis in muscle (Zeitz et al. 2019; Ghavi et al. 2020). This regulation was significant for muscle growth, because the ratio between protein synthesis and catabolism determined the actual rate of protein deposition in muscle (Powell 2016). In this study, the enhancement of protein synthesis in vivo was also observed with increasing dietary Met levels, which was manifested in the increase of the relative weight of breast muscle, this response providing a significant fit to a cubic model: $Y = 108.2 - 363.4X + 704.5X^2 - 388.4X^3$, where Y is the relative weight of breast muscle and X is the dietary Met level. Muscle accretion includes two types: myofibrillar hypertrophy and sarcoplasmic hypertrophy. As mentioned by Zhai et al. (2012, 2016), extra Met supplementation induced the increase of protein deposition in muscle, thus increasing the relative weight of breast muscle, which was related to sarcoplasm hypertrophy. Sarcoplasmic hypertrophy would lead to a decrease of myofiber density and an increase of myofiber cross-sectional area (Zatsiorsky and Kraemer 2006). According to Sahebi-Ala et al. (2021), Met as a donor of sulfur group was beneficial to enhance the diameter of myofiber. In addition, Duclos et al. (2007) reported that the average myofiber diameter in birds with higher body weight was larger than those with slower growth. The increase in myofiber per unit area corresponds to the decrease in its density. In the present study, we observed that increasing dietary Met levels could increase the diameter of myofiber and decrease the density of myofiber, which was direct evidence of sarcoplasmic hypertrophy. Similarly, Sahebi-Ala et al. (2021) noted that increasing dietary Met levels could increase diameter of myofiber and the relative weight of breast muscle in broiler chicks. Therefore, we considered that increasing dietary Met levels promotes the protein synthesis in muscle, resulting in sarcoplasm hypertrophy, which was manifested in the increase of myofiber diameter and the decrease of myofiber density, thus improving the relative weight of breast muscle. In the present study, density of myofiber decreased as dietary Met level increased, this response providing a significant fit to a linear model: $Y = 44.41 - 9.27X$, where Y is the myofiber density and X is the dietary Met level. In addition, diameter of myofiber increased as dietary Met level increased, this response providing a significant fit to a cubic model: $Y = 106.2 - 165.3X + 317.2X^2 - 168.8X^3$, where Y is the myofiber diameter and X is the dietary Met level.

It has been reported that the relative weight of abdominal fat has a negative response to the increase of dietary Met levels (Chattopadhyay et al. 2006; Majdeddin et al. 2019). However, in this study, the relative weight of abdominal fat increased with the increase of Met levels in the diet. Applegate (2008) noted that if the ratio of amino acid provided to the demand of birds was inappropriate, excess amino acids will be deaminated and likely used as a source of energy. The increase of serum uric acid concentration indicated the occurrence of excessive deamination of amino acid in vivo (Karami et al. 2018; Ghavi et al. 2020). In addition, Del Vesco et al. (2013) mentioned that increasing dietary Met levels could decrease the expression of uncoupling protein in the muscle, which results in the energy being used for ATP synthesis instead of heat production (Ledesma et al. 2002; Cannon et al. 2006). This probably means that the energy supply was increased in the body. In poultry, the increase of energy supply usually corresponds with the deposition of fat in vivo (Wen et al. 2017). Therefore, we considered that extra Met supplementation resulted in the occurrence of excessive deamination of Met, which was manifested in the increase of serum uric acid concentration. This probably led to the increase of energy supply in vivo, thus promoting deposition of abdominal fat. On the other hand, liver is the site for lipid metabolism; the variation of its weight reflects the level of metabolic activity intensity (Liu et al. 2014; Jones 2016). In this study, increasing dietary Met levels resulted in the increase of relative weight of liver, which probably means that the increase of the lipid synthesis. We also considered that the increase in the relative weight of liver was helpful to increase the relative weight of abdominal fat. The relative weight of liver and abdominal fat increased as dietary Met level increased, this response providing a significant fit to linear models, respectively: $Y = 17.52 + 6.70X$, where Y is the relative weight of liver and X is the dietary Met level; and $Y = 4.89 + 8.73X$, where Y is the relative weight of abdominal fat and X is the dietary Met level.

Immune organs in poultry mainly include thymus, spleen, and bursa of fabricius (Ruan et al. 2017). The reduction of relative weight of immune organs represents immunosuppression, while the increase of the immune organs means immune enhancement (Iftikhar et al. 2012). However, in the present study, increasing dietary Met levels did not affect the relative weight of thymus, spleen, and bursa of fabricius. The requirement of Met level for optimal immunity in birds has been reported to be higher than its requirement for optimal

growth (Shini et al. 2005; Hassanein and El-Sagheer 2006). As the results observed in this study, increasing dietary Met levels did not generate positive effects on the immune status of geese through increasing the relative weight of immune organs, and also did not generate any negative effects.

Serum biochemical parameters, such as total protein, albumin, and uric acid, are indicators of physiological and nutritional status of birds (Reda et al. 2020; Wu et al. 2021). The concentrations of serum total protein reflect the protein synthesis capacity in vivo (Wu et al. 2021). In this study, the serum total protein concentration increased as dietary Met level increased, this response providing a significant fit to a linear model: $Y = 1.20 + 0.09X$, where Y is the serum total protein concentration and X is the dietary Met level. This indicated that increasing dietary Met levels could promote protein synthesis in vivo. The concentration of serum albumin was mainly affected by the protein intake (Wada et al. 2018). Strategies of increasing feed intake could promote protein intake in birds (Kareem 2017). It has been reported that the reduction of protein intake led to the decrease of serum albumin concentration in birds (Emadi et al. 2010). In this study, although the intake of Met increased with the increase of dietary Met supplementation the serum albumin concentration has not fluctuated, which probably indicated that the concentration of serum albumin in geese was not sensitive to the variation of dietary Met levels. Uric acid is the product of amino acid catabolism (Zarghi et al. 2020). The increase of serum uric acid production means the increase of amino acid catabolism (Wen et al. 2014; Sun et al. 2020). It is usually used as an accurate indicator for determining the amino acid requirement (Donsbough 2008). As the result observed in this study, feeding geese with 1.00% Met-containing diet had the highest serum uric acid concentration among all groups, this response providing a significant fit to a linear model: $Y = 266.67 + 150.53X$, where Y is the serum uric acid concentration and X is the dietary Met level. This indicated that the Met supplementation in 1.00% was excessive for geese, but it was still safe, because the feed intake as a Met toxicity indicator did not decline.

In addition, uric acid, like SOD, can also be used as an effective antioxidant (Mahmoudi et al. 2018) to improve antioxidant status in vivo (Ognik and Krauze 2016). MDA, as the final product of lipid peroxidation, is a good indicator representing oxidative damage (Kalvandi et al. 2019; Todorovic et al. 2019). It has been reported that increasing dietary Met levels could improve antioxidant status of birds through increasing serum uric acid and SOD concentrations and decreasing serum MDA concentration (Swennen et al. 2011; Wen et al. 2014; Kalvandi et al. 2019). However, in this study, increasing dietary Met levels did not affect the serum SOD and MDA concentrations, which indicated that increasing dietary Met levels did not induce oxidative reaction. According to Dahiya et al. (2007), the requirement of Met level for modulating the concentrations of the antioxidant enzymes in birds was higher than its requirement for optimal growth and G:F. Therefore, we considered that the increase of dietary Met levels did not impair the antioxidant status in geese, but it may be beneficial to the antioxidant status through increasing serum uric acid concentration.

Additionally, it should be noted that the Met requirement of goose evaluated by the studies of Nitsan et al. (1983) and Leclerq et al. (1987) was lower than our study; however, it did not lead to the impairment of performance for geese. The same is true for the recent investigation conducted by the goose breeding company (https://www.dpi.nsw.gov.au/) and the study of Wang et al. (2010). To better understand the Met requirements of geese, more research is needed, and the research needs to be conducted in different breeds and growth stages.

Conclusion

The results observed in this study indicated that the levels of Met (0.25%, 0.50%, 0.75%, and 1.00% Met, as-fed basis) used in this study could be regarded as a tolerable excessive level. In addition, increasing dietary Met levels could promote protein synthesis in vivo, which was manifested in the increase of serum total protein concentration, thus increasing the relative weight of breast muscle and being beneficial to the improvement of growth performance. We also observed that the increase of myofiber diameter and the decrease of myofiber density were related to the increase of dietary Met levels; this provided direct evidence for the relationship between the increase of relative weight of breast muscle and sarcoplasmic hypertrophy caused by the increase of dietary Met levels. However, increasing dietary Met levels led to the increase in the relative weight of liver and the occurrence of excessive deamination of Met, which was manifested in the increase in serum uric acid concentration, this probably being the reason for the increase of the relative weight of abdominal fat. In addition, increasing dietary Met levels may be beneficial to the improvement of antioxidant status in geese through increasing serum uric acid concentration. The appropriate level of dietary Met to maximize the growth performance and myofiber traits in geese was 0.75%.

Article information

History dates

Received: 8 October 2021
Accepted: 2 July 2022
Accepted manuscript online: 26 October 2022
Version of record online: 19 December 2022

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Competing interests

The authors declare no financial of personal conflicts of interest.

Funding information

There was no funding for this submission.

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