Effects of Glutamine on Growth Performance, Serum Biochemical Indices, and Antioxidant Capacity of Mink in Summer (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of glutamine (Gln) on growth performance, serum biochemical indices, and antioxidant capacity of minks during summer. One hundred and sixty growing minks were selected and randomly divided into 5 groups with 32 minks per group (16 males and 16 females), housed individually in cages. Minks in the control group were fed a basal diet without Gln supplementation, while those in the experimental groups were fed experimental diets supplemented with 0.2%, 0.4%, 0.6%, and 0.8% Gln based on the basal diet. The experimental period lasted for 8 weeks. The results showed that dietary supplementation with 0.4% and 0.6% Gln significantly increased the average daily feed intake of female minks ($P<0.05$); dietary supplementation with 0.4% Gln highly significantly increased the average daily gain of minks ($P<0.01$), and significantly decreased the feed conversion ratio ($P<0.05$). Dietary Gln supplementation had no significant effect on serum glucose content and creatine kinase activity in minks ($P>0.05$), but supplementation with 0.4% and 0.6% Gln significantly increased serum total protein content ($P<0.05$) and significantly decreased serum urea nitrogen content ($P<0.05$). Dietary supplementation with 0.2% and 0.4% Gln significantly increased serum hydroxyl radical scavenging capacity ($P<0.05$), but had no significant effect on serum superoxide anion scavenging capacity ($P>0.05$); dietary supplementation with 0.4% and 0.6% Gln significantly decreased serum malondialdehyde content ($P<0.05$), highly significantly increased serum total antioxidant capacity ($P<0.01$), and significantly increased serum glutathione peroxidase activity ($P<0.05$). Based on comprehensive analysis of all indices, the optimal supplementation level of Gln in the diet of growing minks under high temperature conditions in summer was 0.4%.

Full Text

Effects of Glutamine on Growth Performance, Serum Biochemical Indices, and Antioxidant Capacity of Minks in Summer

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Abstract: This experiment investigated the effects of dietary supplementation with different levels of glutamine (Gln) on growth performance, serum biochemical indices, and antioxidant capacity of minks during summer. One hundred sixty growing minks were selected and randomly divided into five groups, with 32 minks per group (16 males and 16 females), housed individually in single cages. The control group was fed a basal diet without Gln, while the experimental groups were fed the basal diet supplemented with 0.2%, 0.4%, 0.6%, and 0.8% Gln, respectively. The experimental period lasted for eight weeks.

The results showed that dietary supplementation with 0.4% and 0.6% Gln significantly increased the average daily feed intake of female minks ($P<0.05$). Supplementation with 0.4% Gln significantly increased the average daily gain of minks ($P<0.01$) and significantly reduced the feed-to-gain ratio ($P<0.05$). Dietary Gln supplementation had no significant effect on serum glucose content or creatine kinase activity ($P>0.05$), but 0.4% and 0.6% Gln significantly increased serum total protein content ($P<0.05$) and significantly decreased serum urea nitrogen content ($P<0.05$). Supplementation with 0.2% and 0.4% Gln significantly improved serum hydroxyl radical scavenging capacity ($P<0.05$) but had no significant effect on superoxide anion scavenging capacity ($P>0.05$). Dietary supplementation with 0.4% and 0.6% Gln significantly reduced serum malondialdehyde content ($P<0.05$), significantly increased serum total antioxidant capacity ($P<0.01$), and significantly enhanced serum glutathione peroxidase activity ($P<0.05$). Based on comprehensive evaluation of all indices, the optimal dietary Gln supplementation level for growing minks under summer high-temperature conditions is 0.4%.

Keywords: mink; glutamine; growth performance; serum biochemical indices; antioxidant capacity

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Introduction

Heat stress is a widespread problem in animal production that causes substantial economic losses annually [1]. With rising global temperatures and the rapid development of China' s mink farming industry, concerns about heat stress in minks have grown. Minks naturally inhabit cold regions of North America and Northern Europe and prefer cool environments while being highly sensitive to

heat. Summer conditions of high temperature and humidity significantly impact mink health and growth. Heat stress not only reduces feed intake but also affects intestinal health and nutrient digestion and absorption, with severe cases leading to heatstroke, heat exhaustion, and even death, posing serious threats to the industry [2].

Research indicates that high-temperature stress readily induces oxidative stress in broilers, decreasing activities of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase, reducing the body' s ability to scavenge free radicals, triggering lipid peroxidation, and compromising antioxidant capacity [3]. Glutamine (Gln) is an essential amino acid for animals under high-temperature stress, playing crucial roles in anti-stress responses, antioxidant defense, and immune enhancement [4]. Hu et al. [5] found that under normal conditions, animals can synthesize Gln endogenously, but during stress, endogenous synthesis cannot meet demand, leading to severe stress responses and decreased immunity. Under heat stress, poultry' s Gln requirement exceeds its synthetic capacity, resulting in reduced antioxidant function and immune performance [6]. Li et al. [7] reported that dietary Gln supplementation significantly improved serum and intestinal antioxidant capacity in heat-stressed broilers. Li et al. [8] found that Gln could alleviate oxidative stress induced by lipopolysaccharide (LPS) in weaned piglets. However, few studies have examined the effects of Gln on heat stress in minks. This experiment supplemented different levels of Gln in the diets of growing minks during summer to investigate its effects on growth performance, serum biochemical indices, and antioxidant parameters, aiming to explore Gln' s impact on antioxidant capacity and provide a basis for alleviating heat stress in minks.

1.1 Experimental Materials and Diets

The Gln used in this experiment was purchased from Wuxi Jinweian Biotechnology Co., Ltd. (food grade, 99% active ingredient content). The composition and nutrient levels of the basal diet are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (DM basis)
Note: DM, CP, EE, ash, Ca, P, Lys, Met, and Cys were measured values, while ME was a calculated value.

1.2 Experimental Animals and Design

One hundred sixty growing short-haired black minks (offspring of American short-haired black and standard minks) were selected after weaning and separation, including 80 males and 80 females with similar body weights within each sex. The minks were randomly divided into five groups of 32 animals each (half male and half female), housed individually in single cages, with each group representing 32 replicates. One group served as the control, fed the basal diet

without Gln, while groups 2–5 were experimental groups fed the basal diet supplemented with 0.2%, 0.4%, 0.6%, and 0.8% Gln, respectively. The experiment ran from July 3 to August 27, 2016, with a one-week pre-trial period and a seven-week formal experimental period.

1.3 Management

The experiment was conducted at a mink farm in Jimo District, Qingdao, Shandong Province. Before the trial, all sheds, cages, and floors were thoroughly cleaned and disinfected. Minks had ad libitum access to water and feed, receiving their respective diets at 07:00 and 17:00 daily. For each feeding, the required Gln was first dissolved in a small amount of water and then thoroughly mixed with the weighed basal diet.

Temperature and humidity in the sheds were recorded using HOBO data loggers (placed 30 cm above the cages). In July, daytime (09:00–17:00) average temperature was 30.1°C, with a maximum of 38.6°C (exceeding 35°C on 10 days), while nighttime average temperature was 25.4°C. Daytime relative humidity averaged 75.5% and nighttime 87.1% (exceeding 95% on 6 days). In August, daytime average temperature was 31.4°C, with a maximum of 39.0°C (exceeding 35°C on 17 days), while nighttime average temperature was 26.8°C. Daytime relative humidity averaged 76.7% and nighttime 89.3% (exceeding 95% on 7 days).

1.4 Blood Collection and Serum Preparation

At the end of the feeding trial, six male and six female minks with body weights close to the group average were randomly selected from each group. Blood samples (3 mL) were collected via toe clipping and centrifuged at 3,000 rpm for 10 minutes at low temperature. The supernatant serum was collected, aliquoted into 1.5 mL centrifuge tubes, and stored at -80°C for subsequent analysis.

1.5.1 Growth Performance Indices

During the experiment, feed intake and leftovers were recorded daily to calculate average daily feed intake (ADFI) for males and females separately. Body weight was measured weekly in the morning after fasting to determine average daily gain (ADG) for each sex. Feed-to-gain ratio was calculated based on ADFI and ADG. Mortality was recorded throughout the trial.

1.5.2 Serum Biochemical Indices

Serum glucose (GLU) content was measured using the glucose oxidase method; total protein (TP) content using the Coomassie brilliant blue method; urea nitrogen (UN) content using the diacetyl monoxime method; and creatine kinase (CK) activity using the colorimetric method. All assays were performed using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.5.3 Serum Antioxidant Indices

Serum hydroxyl radical (· OH) scavenging capacity was measured using the colorimetric method; superoxide anion radical (O ·) scavenging capacity using the chemical colorimetric method; malondialdehyde (MDA) content using the thiobarbituric acid (TBA) method; total antioxidant capacity (T-AOC) using the colorimetric method; superoxide dismutase (SOD) activity using the hydroxylamine method; glutathione peroxidase (GSH-Px) activity using the colorimetric method; and catalase (CAT) activity using the ammonium molybdate method. All assays were performed using commercial kits from Nanjing Jiancheng Bioengineering Institute.

1.6 Data Processing and Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 17.0 software, followed by LSD multiple comparisons. Results are expressed as "mean ± standard deviation." Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$.

Results

2.1 Effects of Gln on Mink Growth Performance

The effects of Gln on male mink growth performance are shown in Table 2 . Average daily feed intake did not differ significantly among groups ($P>0.05$). Average daily gain in all experimental groups was higher than in the control group, with the 0.4% Gln group being significantly higher than the control group ($P<0.01$) and both the 0.2% and 0.4% Gln groups being significantly higher than the 0.6%, 0.8%, and control groups ($P<0.05$). The feed-to-gain ratio in the control group was significantly higher than in all experimental groups ($P<0.05$). Among experimental groups, the 0.4% Gln group had the lowest feed-to-gain ratio, which was significantly lower than the 0.8% and 0.6% Gln groups ($P<0.05$). During the trial, one male mink died in both the control and 0.2% Gln groups, with no mortality in other groups.

Table 2 Effects of Gln on growth performance of male minks
Note: In the same row, values with the same small letter or no letter superscripts indicate no significant difference ($P>0.05$), different small letters indicate significant difference ($P<0.05$), and different capital letters indicate highly significant difference ($P<0.01$). The same applies below.

The effects of Gln on female mink growth performance are presented in Table 3 . Average daily feed intake was significantly higher in the 0.4% and 0.6% Gln groups compared to the 0.2%, 0.8%, and control groups ($P<0.05$), with no significant differences among other groups ($P>0.05$). All experimental groups showed higher average daily gain than the control group, with the 0.4% Gln

group being the highest and significantly higher than the 0.2%, 0.8%, and control groups (P<0.01), while the 0.6% Gln group was also significantly higher than these groups (P<0.05). The feed-to-gain ratio in the control group was significantly higher than in all experimental groups (P<0.05), with the 0.4% Gln group having the lowest ratio, significantly lower than the 0.2% and 0.8% Gln groups (P<0.05). Mortality occurred in one female mink each in the control, 0.6% Gln, and 0.8% Gln groups, with no deaths in the other two groups.

Table 3 Effects of Gln on growth performance of female minks

2.2 Effects of Gln on Serum Biochemical Indices

Table 4 shows the effects of Gln on serum biochemical indices in male minks. No significant differences were observed among groups in serum glucose, total protein, urea nitrogen content, or creatine kinase activity (P>0.05), indicating that dietary Gln supplementation did not significantly affect these biochemical parameters in male minks.

Table 4 Effects of Gln on serum biochemical indices of male minks

The effects of Gln on serum biochemical indices in female minks are shown in Table 5 . Dietary Gln supplementation had no significant effect on serum glucose content or creatine kinase activity (P>0.05) but significantly influenced serum total protein and urea nitrogen content (P<0.05). Serum total protein in all experimental groups was higher than in the control group, with the 0.4% Gln group being significantly higher than the 0.2% Gln and control groups (P<0.05). Serum urea nitrogen content in the control group was significantly higher than in the 0.4%, 0.6%, and 0.8% Gln groups (P<0.05), with no significant differences among other groups (P>0.05).

Table 5 Effects of Gln on serum biochemical indices of female minks

2.3 Effects of Gln on Serum Antioxidant Indices

The effects of Gln on serum antioxidant indices in male minks are presented in Table 6 . Hydroxyl radical scavenging capacity in all experimental groups was higher than in the control group, with the 0.2% and 0.4% Gln groups being significantly higher than the 0.8% Gln and control groups (P<0.05). No significant differences were observed among groups in superoxide anion scavenging capacity (P>0.05). Serum MDA content in the control, 0.2% Gln, and 0.8% Gln groups was significantly higher than in the 0.4% and 0.6% Gln groups (P<0.05). Total antioxidant capacity in all experimental groups was higher than in the control group, with the 0.4% and 0.6% Gln groups being highly significantly higher than the control and 0.2% Gln groups (P<0.01), the 0.8% Gln group being highly significantly higher than the control group (P<0.01), and the 0.8% Gln group being significantly higher than the 0.2% Gln group (P<0.05). No significant differences were found among groups in superoxide dismutase activity (P>0.05). Glutathione peroxidase activity in all experimental groups was

higher than in the control group, with the 0.2%, 0.4%, and 0.6% Gln groups being significantly higher than the control group ($P<0.05$) and the 0.4% Gln group being significantly higher than the 0.8% Gln group ($P<0.05$). Catalase activity in all experimental groups was higher than in the control group, with the 0.4% Gln group being significantly higher than the control group ($P<0.05$).

Table 6 Effects of Gln on serum antioxidant indices of male minks

The effects of Gln on serum antioxidant indices in female minks are shown in Table 7 . Hydroxyl radical scavenging capacity in all experimental groups was higher than in the control group, with the 0.4% Gln group being significantly higher than the control, 0.2% Gln, and 0.8% Gln groups ($P<0.05$) and the 0.6% Gln group being significantly higher than the control group ($P<0.05$). No significant differences were observed among groups in superoxide anion scavenging capacity ($P>0.05$). Serum MDA content in the control group was significantly higher than in the 0.2%, 0.4%, and 0.6% Gln groups ($P<0.05$), while the 0.8% Gln group was significantly higher than the 0.4% and 0.6% Gln groups ($P<0.05$). Total antioxidant capacity in all experimental groups was highly significantly higher than in the control group ($P<0.01$), with no significant differences among experimental groups ($P>0.05$). Superoxide dismutase activity in the 0.6% Gln group was significantly higher than in the 0.2% Gln, 0.8% Gln, and control groups ($P<0.05$). Glutathione peroxidase activity in the 0.4% and 0.6% Gln groups was significantly higher than in the 0.2% Gln, 0.8% Gln, and control groups ($P<0.05$), with the 0.8% Gln group also being significantly higher than the control group ($P<0.05$). No significant differences were found among groups in catalase activity ($P>0.05$).

Table 7 Effects of Gln on serum antioxidant indices of female minks

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Discussion

3.1 Effects of Gln on Growth Performance of Minks in Summer

High environmental temperatures reduce feed intake and growth rate while impairing feed conversion efficiency in livestock and poultry [1,3-4]. Minks naturally inhabit cold regions and are highly sensitive to heat. During our experiment, average shed temperatures in July and August exceeded 30°C, with maximum temperatures reaching 39°C and average relative humidity above 75%, conditions sufficient to induce heat stress and affect feed intake and growth. Numerous studies have shown that dietary Gln supplementation improves growth performance in heat-stressed animals. Dong et al. [9] reported that appropriate Gln supplementation in broiler diets increased feed intake, daily gain, and reduced feed-to-gain ratio. Lu et al. [10] found that Gln supplementation under heat stress improved broiler feed intake and daily gain. This is related to Gln' s unique physiological functions—under high temperatures, animals require more Gln than they can synthesize endogenously, and exogenous Gln is needed

to maintain normal metabolism and immune function for healthy growth [3]. Our results similarly demonstrate that under summer high-temperature conditions, dietary Gln supplementation increased average daily gain in both male and female growing minks, increased average daily feed intake in females, and reduced feed-to-gain ratio. As Gln supplementation increased, weight gain improved, peaking at 0.4% Gln and then declining. These findings indicate that 0.4% dietary Gln is sufficient to alleviate the adverse effects of high temperature on feed intake and growth, with further increases providing no additional benefits.

3.2 Effects of Gln on Serum Biochemical Indices of Minks in Summer

Reduced feed intake during hot seasons leads to insufficient nutrient intake, inevitably altering metabolism and accelerating the breakdown of body reserves to meet nutritional needs, thereby changing blood biochemical parameters. Liu et al. [11] reported that high temperatures decreased serum total protein and increased serum urea nitrogen in broilers. Liu et al. [12] found that blood glucose remained stable in heat-stressed chickens. Huang et al. [13] demonstrated that dietary Gln significantly increased blood total protein and decreased urea nitrogen in broilers under high temperature, without affecting blood glucose. Our results align with these findings, showing no significant effects of Gln on serum glucose or creatine kinase activity, suggesting that Gln helps maintain metabolic homeostasis. We also found that Gln increased serum total protein and decreased urea nitrogen in female minks, likely because minks have high protein requirements during growth, and Gln improved protein utilization, increased protein synthesis, and reduced urea nitrogen, thereby mitigating heat stress effects. However, Gln did not significantly affect these parameters in males, possibly due to sex differences. Based on serum biochemical indices, 0.2% Gln appears to be an effective supplementation level.

3.3 Effects of Gln on Serum Antioxidant Enzyme Activity of Minks in Summer

Key antioxidant enzymes include glutathione peroxidase, catalase, and superoxide dismutase. Research shows that Gln affects the activity of these enzymes, promotes glutathione (GSH) synthesis, and maintains reduced GSH levels [14]. High summer temperatures enhance oxidative stress, increasing reactive oxygen species that require elevated antioxidant enzyme activity for neutralization. Huang et al. [15] reported that 0.5% and 0.8% dietary Gln significantly increased glutathione peroxidase activity and reduced MDA content in 30-day-old yellow-feathered broilers, without affecting SOD activity. Li et al. [7] found that Gln supplementation significantly increased serum glutathione peroxidase, catalase, and SOD activities in heat-stressed broilers, with 1.6% Gln being optimal. Our results demonstrate that appropriate Gln levels significantly increased glutathione peroxidase and catalase activities in male minks and SOD and glutathione peroxidase activities in females, indicating that Gln can enhance an-

tioxidant enzyme activity in summer. The optimal Gln level was 0.2%–0.4% for males and 0.4%–0.6% for females. For practical production, a uniform supplementation level of 0.4% Gln is recommended for both sexes.

3.4 Effects of Gln on Serum Free Radical Scavenging Capacity of Minks in Summer

Reactive oxygen species, particularly hydroxyl and superoxide anion radicals, are primary free radicals that can cause various pathological conditions and threaten animal health. Under normal conditions, free radical production and elimination are in dynamic equilibrium, but under stress conditions like high temperature, scavenging capacity decreases and free radicals accumulate, causing health damage [16]. Our study found that dietary Gln improved serum hydroxyl radical scavenging capacity in both male and female minks during summer, indicating that heat stress induces oxidative stress and that Gln exerts beneficial effects on antioxidant status. The optimal Gln supplementation level for improving free radical scavenging capacity is 0.4% for both sexes.

3.5 Effects of Gln on Serum MDA Content and Total Antioxidant Capacity of Minks in Summer

Malondialdehyde, a product of lipid peroxidation, is a primary marker of oxidative stress [17]. Total antioxidant capacity is a comprehensive indicator that reflects overall free radical metabolism status and the antioxidant system' s compensatory response to external stimuli [18]. Li et al. [7] reported that dietary Gln significantly increased serum total antioxidant capacity and decreased MDA content in heat-stressed broilers. Our results similarly show that Gln supplementation significantly increased serum total antioxidant capacity and reduced MDA content in both male and female minks, demonstrating that Gln can enhance antioxidant capacity and mitigate the adverse effects of summer heat stress. The optimal Gln supplementation level for both sexes is 0.4%–0.6%.

Conclusion

Under summer high-temperature conditions, dietary Gln supplementation improved average daily gain, reduced feed-to-gain ratio, enhanced protein metabolism, and increased antioxidant capacity in minks. Based on comprehensive evaluation of growth performance, serum biochemical indices, and antioxidant parameters, the optimal dietary Gln supplementation level for minks in summer is 0.4%.

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