Effects of Glutamine on Growth Performance, Serum Biochemical Parameters, and Antioxidant Capacity of Minks During Summer: A Postprint

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Abstract

This study aimed to investigate the effects of dietary supplementation with different levels of glutamine (Gln) on growth performance, serum biochemical parameters, and antioxidant capacity in mink during summer. One hundred and sixty growing mink were selected and randomly allocated into five groups, with 32 mink per group (16 males and 16 females), housed individually in cages. The control group was fed a basal diet without Gln supplementation, while the experimental groups were fed test 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 mink ($P<0.05$). Dietary supplementation with 0.4% Gln extremely significantly increased the average daily gain of mink ($P<0.01$) and significantly decreased the feed conversion ratio ($P<0.05$). Dietary Gln supplementation had no significant effect on serum glucose concentration or creatine kinase activity ($P>0.05$), but supplementation with 0.4% and 0.6% Gln significantly increased serum total protein concentration ($P<0.05$) and significantly decreased serum urea nitrogen concentration ($P<0.05$). Dietary supplementation with 0.2% and 0.4% Gln significantly enhanced 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 concentration ($P<0.05$), extremely significantly increased serum total antioxidant capacity ($P<0.01$), and significantly increased serum glutathione peroxidase activity ($P<0.05$). Based on comprehensive evaluation of all parameters, the optimal dietary supplementation level of Gln for growing mink under summer high-temperature conditions is 0.4%.

Full Text

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

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Abstract: This experiment investigated the effects of dietary glutamine (Gln) supplementation at varying levels on growth performance, serum biochemical parameters, and antioxidant capacity in minks during summer. One hundred and sixty growing minks were randomly allocated into five groups, each comprising 32 minks (16 males and 16 females) with individual housing. The control group received a basal diet without Gln supplementation, while the experimental groups were fed the basal diet supplemented with 0.2%, 0.4%, 0.6%, or 0.8% Gln. The trial lasted for eight weeks.

The results demonstrated that dietary supplementation with 0.4% and 0.6% Gln significantly increased average daily feed intake in female minks ($P<0.05$). Supplementation at 0.4% Gln markedly elevated average daily gain ($P<0.01$) and significantly reduced feed-to-gain ratio ($P<0.05$) across all minks. While serum glucose concentration and creatine kinase activity remained unaffected by Gln supplementation ($P>0.05$), dietary inclusion of 0.4% and 0.6% Gln significantly increased serum total protein content ($P<0.05$) and decreased serum urea nitrogen concentration ($P<0.05$). Supplementation with 0.2% and 0.4% Gln significantly enhanced serum hydroxyl radical scavenging capacity ($P<0.05$), though no significant effects were observed on superoxide anion scavenging capacity ($P>0.05$). Additionally, 0.4% and 0.6% Gln supplementation significantly reduced serum malondialdehyde content ($P<0.05$), substantially elevated total antioxidant capacity ($P<0.01$), and increased glutathione peroxidase activity ($P<0.05$). Based on comprehensive evaluation of all measured parameters, the optimal dietary Gln supplementation level for growing minks under summer heat stress conditions is 0.4%.

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

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Introduction

Heat stress represents a pervasive challenge in animal production, causing substantial economic losses annually [1]. With rising global temperatures and the rapid expansion of mink farming in China, heat stress in minks has emerged as a critical concern. Minks are naturally adapted to cool climates and are highly susceptible to heat. Summer conditions characterized by high temperature and

humidity significantly compromise mink health and growth, reducing feed intake, impairing intestinal function and nutrient absorption, and in severe cases causing heatstroke and mortality, thereby posing serious threats to the industry [2].

Research indicates that heat stress induces oxidative stress in broilers by decreasing activities of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase, impairing free radical scavenging capacity and triggering lipid peroxidation [3]. Glutamine (Gln) becomes an essential amino acid under heat stress, playing vital roles in anti-stress responses, antioxidant defense, and immune enhancement [4]. Hu et al. [5] reported that while animals can synthesize Gln endogenously under normal conditions, this capacity becomes insufficient during stress, leading to severe stress responses and compromised immunity. In heat-stressed poultry, Gln requirements exceed synthetic capacity, resulting in diminished antioxidant function and immune performance [6]. Li et al. [7] demonstrated that dietary Gln supplementation significantly improved serum and intestinal antioxidant capacity in heat-stressed broilers. Li et al. [8] found that Gln alleviated oxidative stress induced by lipopolysaccharide (LPS) in weaned piglets. However, research on Gln effects in heat-stressed minks remains scarce. This study examined the impacts of varying dietary Gln levels on growth performance, serum biochemical parameters, and antioxidant indices in growing minks during summer to elucidate its effects on antioxidant capacity and provide evidence for mitigating heat stress in mink production.

Materials and Methods

1.1 Experimental Materials and Diets

Food-grade glutamine (99% purity) was purchased from Wuxi Jinweian Biotechnology Co., Ltd. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (DM basis)

Ingredients	Content (%)	Nutrient levels	Content
Fish steak	[value]	Dry matter (DM)	[value]
Sea fish	[value]	Metabolizable energy (ME) (MJ/kg)	[value]
Chicken gastric stomach	[value]	Crude protein (CP)	[value]
Expanded corn	[value]	Crude fat (EE)	[value]
Chicken liver	[value]	Crude ash	[value]
Peanut cake	[value]	Lysine (Lys)	[value]
Egg	[value]	Methionine (Met)	[value]
Chicken head	[value]	Cysteine (Cys)	[value]
Chicken intestine	[value]		
Water	[value]		
Total	**100.00**		

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 and sixty growing short-haired black minks (offspring of American short-haired black and standard mink crossbreeds) post-weaning were selected, including 80 males and 80 females with similar body weights within each sex. The minks were randomly assigned to five groups of 32 animals each (16 males and 16 females per group) with individual housing, resulting in 32 replicates per group. One group served as the control, receiving the basal diet without Gln, while the remaining four experimental groups received the basal diet supplemented with 0.2%, 0.4%, 0.6%, or 0.8% Gln. The trial was conducted from July 3 to August 27, 2016, comprising a one-week adaptation period followed by a seven-week experimental period.

1.3 Feeding Management

The experiment was conducted at a mink farm in Jimo District, Qingdao, Shandong Province. Prior to 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, Gln was first dissolved in a small amount of water and then thoroughly mixed with the weighed basal diet.

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

1.4 Blood Collection and Serum Preparation

At the conclusion of the feeding trial, six male and six female minks with body weights closest to the group mean 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 serum was harvested, aliquoted into 1.5 mL tubes, and stored at -80°C until analysis.

1.5 Measurement Indicators

1.5.1 Growth Performance Daily feed provision and refusal were recorded to calculate average daily feed intake (ADFI) for males and females separately. Body weight was measured weekly after overnight fasting to determine average

daily gain (ADG). Feed-to-gain ratio (F:G) was calculated from ADFI and ADG. Mortality was recorded throughout the trial.

1.5.2 Serum Biochemical Parameters Serum glucose (GLU) was determined using the glucose oxidase method, total protein (TP) by the Coomassie brilliant blue method, urea nitrogen (UN) by the diacetyl monoxime method, and creatine kinase (CK) activity by colorimetric assay. All kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.5.3 Serum Antioxidant Parameters Serum hydroxyl radical (·OH) scavenging capacity, superoxide anion (O ·) scavenging capacity, malondialdehyde (MDA) content (by thiobarbituric acid method), total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity (by hydroxylamine method), glutathione peroxidase (GSH-Px) activity, and catalase (CAT) activity (by ammonium molybdate method) were measured 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 post-hoc tests for multiple comparisons. Results are expressed as means ± standard deviation. Statistical significance was declared at $P<0.05$ and highly significant at $P<0.01$.

Results

2.1 Effects of Gln on 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 ($P>0.05$). However, average daily gain was higher in all treatment groups compared to the control, with the 0.4% Gln group showing highly significant improvement ($P<0.01$). Both the 0.2% and 0.4% Gln groups exhibited significantly higher ADG 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 treatment groups ($P<0.05$), with the 0.4% Gln group achieving the lowest ratio, significantly lower than the 0.8% and 0.6% groups ($P<0.05$). Mortality occurred in one male mink each in the control and 0.2% Gln groups, with no deaths in other groups.

Table 2 Effects of Gln on growth performance of male minks

Item	Gln supplemental level (%)	P-value
	0 (Control)	0.2
ADFI (g)	198.16±37.40	202.17±36.81
ADG (g)	13.74±0.55Bb	14.99±0.90ABa
F:G	14.42±0.53a	13.48±0.31d

Item	Gln supplemental level (%)	P-value
Mortality (%)	3.13	3.13

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 other significant differences. All treatment groups showed higher ADG than the control, with the 0.4% Gln group achieving the highest gain, significantly greater than the 0.2%, 0.8%, and control groups (P<0.01), and the 0.6% group also significantly higher than these groups (P<0.05). The feed-to-gain ratio in the control group was significantly higher than all treatment groups (P<0.05), with the 0.4% Gln group showing the lowest ratio, significantly lower than the 0.2% and 0.8% groups (P<0.05). Mortality occurred in one female mink each in the control, 0.6%, and 0.8% Gln groups.

Table 3 Effects of Gln on growth performance of female minks

Item	Gln supplemental level (%)	P-value
	0 (Control)	0.2
ADFI (g)	142.33±11.69b	140.90±13.11b
ADG (g)	5.62±0.71Bb	5.95±0.59Bb
F:G	25.32±0.57a	23.68±0.41c
Mortality (%)	3.13	0

2.2 Effects of Gln on Serum Biochemical Parameters

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

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

Item	Gln supplemental level (%)	P-value
	0 (Control)	0.2
GLU (mmol/L)	8.48±1.22	7.98±0.17
TP (g/L)	78.55±2.17	79.41±0.68
UN (mmol/L)	13.04±0.45	11.76±0.43

Item	Gln supplemental level (%)	P-value
CK (U/L)	12.01±0.80	12.61±0.35

The effects on female minks are presented in Table 5 . While glucose and creatine kinase remained unaffected (P>0.05), significant effects were observed on total protein and urea nitrogen (P<0.05). All treatment groups showed higher total protein than the control, with the 0.4% Gln group significantly exceeding the 0.2% and control groups (P<0.05). Serum urea nitrogen in the control group was significantly higher than in the 0.4%, 0.6%, and 0.8% Gln groups (P<0.05).

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

Item	Gln supplemental level (%)	P-value
	0 (Control)	0.2
GLU (mmol/L)	8.29±0.70	8.64±0.61
TP (g/L)	78.13±1.25c	79.04±0.21bc
UN (mmol/L)	12.80±0.64a	11.97±0.25ab
CK (U/L)	11.57±0.81	12.00±0.21

2.3 Effects of Gln on Serum Antioxidant Parameters

The effects on male minks are summarized in Table 6 . Hydroxyl radical scavenging capacity was higher in all treatment groups than the control, with significant improvements in the 0.2% and 0.4% Gln groups compared to the 0.8% and control groups (P<0.05). No significant differences were observed in superoxide anion scavenging capacity (P>0.05). Serum malondialdehyde content in the control, 0.2%, and 0.8% Gln groups was significantly higher than in the 0.4% and 0.6% groups (P<0.05). Total antioxidant capacity was elevated in all treatment groups, with the 0.4% and 0.6% groups showing highly significant increases over the control and 0.2% groups (P<0.01), and the 0.8% group also significantly higher than both control and 0.2% groups (P<0.01 and P<0.05, respectively). Superoxide dismutase activity did not differ significantly among groups (P>0.05). Glutathione peroxidase activity was higher in all treatment groups than the control, with significant elevations in the 0.2%, 0.4%, and 0.6% groups (P<0.05), and the 0.4% group significantly higher than the 0.8% group (P<0.05). Catalase activity was also higher in all treatment groups, with the 0.4% Gln group significantly exceeding the control (P<0.05).

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

Item	Gln supplemental level (%)	P-value
	0 (Control)	0.2

Item	Gln supplemental level (%)	P-value
Restraining ·OH ability (U/mL)	587.09±14.90	658.63±33.12a
O · resistance ability (U/L)	776.12±37.80	778.07±41.04
MDA (nmol/mL)	39.30±0.83a	37.63±0.63ab
T-AOC (U/mL)	13.70±1.60Cb	14.91±1.25BCb
SOD (U/mL)	120.09±4.56	120.70±7.15
GSH-Px (U/mL)	534.3±61.0c	724.8±129.5ab
CAT (U/mL)	38.33±0.79b	39.22±0.66ab

The effects on female minks are shown in Table 7 . Hydroxyl radical scavenging capacity was elevated in all treatment groups compared to the control, with the 0.4% Gln group significantly higher than the control, 0.2%, and 0.8% groups ($P<0.05$), and the 0.6% group significantly higher than the control ($P<0.05$). Superoxide anion scavenging capacity did not differ among groups ($P>0.05$). Serum malondialdehyde content in the control group was significantly higher than in the 0.2%, 0.4%, and 0.6% groups ($P<0.05$), while the 0.8% group was significantly higher than both the 0.4% and 0.6% groups ($P<0.05$). Total antioxidant capacity was highly significantly increased in all treatment groups compared to the control ($P<0.01$), with no differences among treatment groups. Superoxide dismutase activity in the 0.6% Gln group was significantly higher than in the 0.2%, 0.8%, and control groups ($P<0.05$). Glutathione peroxidase activity was significantly elevated in the 0.4% and 0.6% groups compared to the 0.2%, 0.8%, and control groups ($P<0.05$), with the 0.8% group also significantly higher than the control ($P<0.05$). Catalase activity did not differ significantly among groups ($P>0.05$).

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

Item	Gln supplemental level (%)	P-value
	0 (Control)	0.2
Restraining ·OH ability (U/mL)	580.48±29.0	617.93±23.0
O · resistance ability (U/L)	777.60±31.0	792.71±23.1
MDA (nmol/mL)	40.11±0.45a	38.23±0.39b

Item	Gln supplemental level (%)	P-value
T-AOC (U/mL)	13.17±1.13	16.06±1.75A
SOD (U/mL)	115.27±6.0	118.42±2.16
GSH-Px (U/mL)	571.8±64.5c	617.5±74.1b
CAT (U/mL)	39.14±1.03	40.18±1.04

Discussion

3.1 Effects on Growth Performance

High ambient temperatures reduce feed intake and impair feed conversion efficiency and growth rate in livestock and poultry [1,3-4]. Minks are naturally adapted to cold climates of North America and Northern Europe and are highly heat-sensitive. During our trial, average shed temperatures exceeded 30°C in both July and August, with maximum temperatures reaching 39°C and average relative humidity above 75%, conditions sufficient to induce heat stress and negatively impact feed intake and growth. Numerous studies have demonstrated that dietary Gln supplementation improves growth performance in heat-stressed animals. Dong et al. [9] reported that appropriate Gln levels in broiler diets enhanced feed intake, daily gain, and reduced feed-to-gain ratio. Lu et al. [10] found that Gln supplementation improved feed intake and daily gain in heat-stressed broilers. These effects relate to Gln' s unique physiological functions: under heat stress, animals' Gln requirements increase while endogenous synthesis becomes inadequate, necessitating exogenous supplementation to maintain normal metabolism and immune function [3]. Our results similarly demonstrate that dietary Gln supplementation during summer increased ADG in both male and female minks, enhanced ADFI in females, and reduced feed-to-gain ratio. Growth rate increased with Gln supplementation up to 0.4%, beyond which it declined. These findings indicate that 0.4% dietary Gln is sufficient to alleviate the adverse effects of heat stress on feed intake and growth in minks, with higher supplementation levels providing no additional benefits.

3.2 Effects on Serum Biochemical Parameters

Reduced feed intake during hot seasons leads to inadequate nutrient intake, accelerating catabolism of body reserves and altering blood biochemical parameters. Liu et al. [11] reported decreased serum total protein and increased urea nitrogen in heat-stressed broilers. Liu et al. [12] found stable blood glucose levels in heat-stressed chickens. Huang et al. [13] demonstrated that Gln supplementation significantly increased serum total protein while reducing urea nitrogen in heat-stressed broilers, without affecting glucose levels. Our results align with these findings, as Gln supplementation did not significantly affect serum glucose or creatine kinase activity in either sex, suggesting Gln helps maintain metabolic homeostasis. We also observed increased serum total protein and decreased urea nitrogen in female minks, likely due to enhanced protein utilization

and increased protein synthesis, thereby mitigating heat stress effects. The lack of significant effects on these parameters in male minks may reflect sex-specific differences. From a biochemical perspective, 0.2% Gln supplementation appears adequate for optimal results.

3.3 Effects on Serum Antioxidant Enzyme Activity

Key antioxidant enzymes include glutathione peroxidase, catalase, and superoxide dismutase. Research shows Gln influences these enzyme activities and promotes glutathione synthesis, maintaining reduced GSH levels [14]. Heat stress elevates reactive oxygen species, requiring enhanced antioxidant enzyme activity to mitigate oxidative damage. Huang et al. [15] reported that 0.5% and 0.8% Gln supplementation significantly increased glutathione peroxidase activity and reduced malondialdehyde content in yellow-feathered broilers at approximately 30 days of age, without affecting SOD activity. Li et al. [7] found that Gln supplementation significantly increased glutathione peroxidase, catalase, and SOD activities in heat-stressed broilers, with 1.6% Gln providing optimal antioxidant effects. Our results demonstrate that appropriate Gln levels significantly increased glutathione peroxidase and catalase activities in male minks and enhanced SOD and glutathione peroxidase activities in females, indicating improved antioxidant enzyme capacity. The optimal Gln level appears to be 0.2–0.4% for males and 0.4–0.6% for females, though 0.4% represents a practical compromise for both sexes in production settings.

3.4 Effects on Serum Free Radical Scavenging Capacity

Reactive oxygen species, particularly hydroxyl and superoxide anion radicals, can cause various pathological conditions. Under normal conditions, free radical generation and elimination maintain dynamic equilibrium, but heat stress impairs scavenging capacity, allowing accumulation of damaging radicals [16]. Our findings show that Gln supplementation enhanced hydroxyl radical scavenging capacity in both sexes during summer, demonstrating that Gln beneficially modulates antioxidant status under heat-induced oxidative stress. The optimal Gln supplementation level for improving free radical scavenging capacity is 0.4% for both male and female minks.

3.5 Effects on Serum MDA Content and Total Antioxidant Capacity

Malondialdehyde, a product of lipid peroxidation, serves as a primary marker of oxidative stress [17]. Total antioxidant capacity reflects the overall antioxidant system status and its compensatory response to external stimuli [18]. Li et al. [7] reported that Gln supplementation significantly increased serum total antioxidant capacity and decreased malondialdehyde content in heat-stressed broilers. Our results similarly demonstrate that Gln supplementation significantly elevated total antioxidant capacity and reduced malondialdehyde levels in both sexes, indicating enhanced antioxidant capacity and mitigation of heat

stress effects. The optimal Gln supplementation level for these parameters is 0.4–0.6% for both male and female minks.

Conclusion

Under summer heat stress conditions, dietary Gln supplementation improved mink growth performance by increasing average daily gain and reducing feed-to-gain ratio, enhanced protein metabolism, and elevated antioxidant capacity. Based on comprehensive evaluation of growth performance, serum biochemical parameters, and antioxidant indices, the optimal dietary Gln supplementation level for minks during summer is 0.4%.

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