Veterinary Integrative Sciences 2026; 24(1): e2026027-1-9. DOI: 10.12982/VIS.2026.027

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)

Research article

Effects of glutamine supplementation on performance, carcass traits, and oxidative stress markers in native crossbred chickens under heat stress conditions

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Abstract

Glutamine is a conditionally essential amino acid known to support energy metabolism, immune function, and antioxidant capacity in poultry. This study aimed to evaluate the effects of dietary glutamine supplementation on growth performance, carcass characteristics, meat quality, and oxidative stress indicators in Pradu Hang dam native crossbred chickens raised in open-sided housing during hot weather conditions. A total of 750 day-old male chicks were allocated to three dietary treatments: control (0% glutamine), 1% glutamine, and 2% glutamine, in a completely randomized design with five replicates per treatment (50 birds/replicate). The trial lasted 12 weeks, from May to July 2024, during which ambient temperature averaged 33.25 °C with relative humidity at 62.5%. Results showed that glutamine supplementation significantly improved final body weight ($P = 0.002$), average daily gain ($P < 0.001$), and feed conversion ratio ($P = 0.021$). Mortality rates decreased in a dose-dependent manner ($P < 0.001$). Carcass traits such as wing, breast, and drumstick weights were significantly increased ($P < 0.05$), while meat protein content was higher in the supplemented groups ($P < 0.05$). Glutamine reduced malondialdehyde (MDA) levels ($P < 0.05$) and increased glutathione (GSH) and glutathione peroxidase (GPx) activity ($P < 0.05$), indicating reduced oxidative stress. No significant differences were observed in cortisol levels. The 1% glutamine level was identified as the optimal dose, offering improved performance with minimal increase in feed cost.

Keywords: Glutamine, Heat stress, Native crossbred chickens, Oxidative stress.

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Article history; *received manuscript: 12 April 2025,*
revised manuscript: 12 May 2025,
accepted manuscript: 24 June 2025,
published online: 15 July 2025,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Heat stress is one of the most critical environmental stressors negatively impacting poultry production, especially in tropical and subtropical regions (Shakeri and Le, 2022). It adversely affects growth performance, feed efficiency, immune response, and oxidative balance in chickens (Sahin et al., 2001). Native crossbred chickens, such as the Pradu Hang dam strain in Thailand, are commonly raised in open housing systems where environmental temperature and humidity are difficult to control (Patcharee et al., 2023). These conditions lead to thermal stress, resulting in reduced productivity and compromised health.

In recent years, nutritional interventions have gained attention as a strategy to mitigate heat stress in poultry. Among various dietary additives, glutamine has emerged as a potential functional amino acid due to its multifaceted roles in cellular metabolism, gut integrity, and antioxidant defense (Li et al., 2007; Wu, 2010). Although glutamine is classified as a non-essential amino acid under normal conditions, it becomes conditionally essential during periods of stress, rapid growth, or immune challenge (Newsholme, 2001).

Glutamine supplementation has been shown to enhance intestinal villi morphology, reduce oxidative damage, and improve immune function in broiler chickens exposed to heat stress (Maiorka et al., 2000; Yi et al., 2005; Dai et al., 2009; Ncho et al., 2023). However, limited data are available on its application in native or crossbred chickens, particularly under semi-intensive management systems prevalent in tropical regions. Given the increasing demand for sustainable poultry production under challenging climates, exploring dietary strategies to improve resilience and performance is crucial. Several biomarkers have been widely used to evaluate heat stress responses in poultry. Malondialdehyde (MDA) is a byproduct of lipid peroxidation and is considered a key indicator of oxidative damage to cellular membranes (Sahin et al., 2001). Glutathione (GSH) is a major intracellular antioxidant, while glutathione peroxidase (GPx) is an enzyme that utilizes GSH to reduce hydrogen peroxide and organic hydroperoxides, thereby protecting cells from oxidative stress (Wu, 2010). Heat stress has been shown to deplete GSH levels and reduce GPx activity in broilers, leading to increased oxidative injury. Additionally, cortisol, a glucocorticoid hormone released through the hypothalamic–pituitary–adrenal (HPA) axis, serves as a physiological marker of systemic stress in birds. Elevated cortisol levels under heat stress are often associated with immunosuppression and reduced productivity (Lin et al., 2006; Abdulkarimi et al., 2019).

This study aimed to investigate the effects of dietary glutamine supplementation on growth performance, carcass traits, and oxidative stress markers in native crossbred chickens reared under naturally hot and humid conditions. The findings are expected to contribute to practical feeding strategies that enhance the welfare and productivity of poultry in tropical environments.

MATERIALS AND METHODS

Experimental Design and Animals

The experiment was conducted at the Poultry Research Facility, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Thailand, during the hot season from May to July 2024. A total of 750 one-day-old male native crossbred chickens (Pradu Hang dam strain) were used. The birds were individually weighed and randomly assigned to one of three dietary treatments in a completely randomized design (CRD) with five replicates per treatment and 50 birds per replicate. All animal procedures were approved by the Institutional Animal Care and Use Committee of Chiang Mai University (Protocol No. AG02001/2567, approved on January 17, 2024).

The dietary treatments were as follows:
Control group: Basal diet without glutamine supplementation (0%)
GLN-1% group: Basal diet supplemented with 1% glutamine
GLN-2% group: Basal diet supplemented with 2% glutamine
The experimental period lasted 12 weeks, with birds raised under open-sided housing conditions simulating commercial semi-intensive tropical production systems.

Housing and Management

All birds were housed in deep-litter floor pens (3 × 4 meters per replicate), with rice husk bedding material. Each pen was equipped with nipple drinkers and hanging feeders. Feed and clean drinking water were provided ad libitum. The birds were not vaccinated during the trial to minimize confounding immunological responses. Ambient temperature and relative humidity were monitored daily using a digital thermo-hygrometer. The average temperature during the study was 33.25 °C with 62.5% relative humidity, indicating moderate to severe heat stress conditions, as defined by Lin et al. (2006) and supported by THI classifications reported in Sahin et al. (2001).

Diet Formulation

The basal diet was formulated to meet or exceed the nutrient requirements of growing chickens according to NRC (1994) and Thai agricultural feed standards. All diets were isocaloric and isonitrogenous. The glutamine used was food-grade L-glutamine (≥99% purity) sourced from a commercial supplier. Feed was prepared weekly and stored in sealed containers. Table 1 presents the ingredients and chemical composition of the basal diet.

Table 1 Ingredients and chemical composition of basal diets.

Ingredients	Percent inclusion		
	0-3 weeks	3-10 weeks	10-12 weeks
Corn	56.00	60.70	61.30
Rice bran	12.00	10.00	15.00
Soybean meal	23.20	18.30	13.60
Fish meal	7.00	6.00	5.00
Leucaena leaf meal	0.00	3.00	3.00
Crushed oyster shell	0.50	0.60	0.60
Dicalcium phosphate	0.70	0.80	0.90
Salt	0.35	0.35	0.35
Premix[1]	0.25	0.25	0.25
Chemical composition analysis (dry matter)			
% Crude protein	20	18	16
ME (Kcal/kg)	2,800	2,700	2,600

[1]Premix = The premix provided following per kg of diet. 36,000 IU vitamin A; 7,200 IU vitamin D3; 10.9 mg; vitamin E; 3.8 mg vitamin K3; 0.37 mg vitamin B1; 7.75 mg vitamin B2; 64 mg nicotinic acid; 10 mg; calcium pantothenate; 0.09 mg vitamin B6; 1,250 mg choline chloride; 10 mg Fe; 20 mg Mn; 20 mg Zn.

Sampling and Data Collection

Growth performance parameters including body weight (BW), average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR) were recorded weekly. Mortality was recorded daily, and birds that died were necropsied to determine cause of death.

At the end of the trial (week 12), 10 birds per treatment group (2 birds per replicate) were randomly selected for slaughter. Carcass traits including carcass weight, dressing percentage, breast weight, drumstick, thigh, and wing weights were measured. Meat samples from the right pectoralis major were collected for proximate composition analysis (moisture, protein, fat, ash) following AOAC (2000) methods.

Blood samples were collected via wing vein prior to slaughter for oxidative stress analysis. Plasma was separated and stored at −20 °C. The following parameters were analyzed:

Malondialdehyde (MDA) – a marker of lipid peroxidation
Glutathione (GSH) – a key antioxidant molecule
Glutathione peroxidase (GPx) activity
Cortisol levels (as a systemic stress indicator)

Analyses were conducted using commercial ELISA kits following manufacturer protocols from Sigma-Aldrich, USA.

Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA) using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). When significant differences were detected ($P < 0.05$), Tukey's post hoc test was used for multiple comparisons among treatment means. Results are expressed as means ± standard error of the mean (SEM). Significance was declared at $P < 0.05$.

RESULTS

Growth Performance

Table 2 summarizes the effects of glutamine supplementation on growth performance of native crossbred chickens. Birds fed 1% and 2% glutamine diets had significantly higher final body weight ($P = 0.002$) and average daily gain ($P < 0.001$) compared to the control group. Feed intake did not differ significantly among treatments ($P > 0.05$), but feed conversion ratio (FCR) was significantly improved in the GLN-1% group ($P = 0.021$). Mortality rate decreased in a dose-dependent manner ($P < 0.001$), with the lowest mortality observed in the GLN-2% group.

Table 2 Effects of Glutamine Supplementation on Growth Performance of heat-stressed native crossbred chickens

Performance (12 weeks)	Control	L-Glu 1%	L-Glu 2%	SEM[1] (pool)	*P-value (<0.05)*
Initial weight; g	44.40	44.16	44.06	0.11	0.504
Weight at 4 weeks; g	456.52^a	501.42^b	505.80^b	6.04	*<0.001*
Weight at 8 weeks; g	1,025.12^a	1,030.76^b	1,035.48^c	1.89	*0.014*
Weight at 12 weeks g	1,349.94^a	1,498.18^b	1,532.98^c	26.45	*0.002*
Cumulative feed intake 0-3 weeks; g/head	444.20	440.40	437.40	2.59	0.598
Cumulative feed intake 0-10 weeks; g/head	2,455.50^a	2,551.75^b	2,546.50^b	15.02	*<0.001*
Cumulative feed intake 0-12 weeks; g/head	4,120.33^a	4,240.00^b	4,337.00^b	33.48	*0.015*
Average daily feed intake; g/head/day (FI)	49.05^a	50.48^b	51.85^b	0.39	*0.014*
Weight gain; g	1,305.54^a	1,454.02^b	1,488.91^b	27.93	*<0.001*
Average daily gain; g/day (ADG)	15.54^a	17.30^b	17.72^b	0.67	*<0.001*
Feed conversion ratio (FCR)	3.16^b	2.92^a	2.88^a	0.45	*0.021*
Mortality rate %	12.00^c	6.00^b	3.20^a	2.67	*<0.001*
Feed cost per gain; Baht/kg (FCG)	56.40^a	60.53^b	68.16^c	2.54	*0.037*

[1]SEM = pooled standard error of the mean, L-Glu = L-Glutamine
[a, b, c] = The means with different superscripts within the same row are significantly different at a statistical level ($P<0.05$).

Carcass Traits

Glutamine supplementation significantly influenced carcass characteristics (Table 3). Birds receiving 1% and 2% glutamine exhibited increased weights of breast ($P = 0.038$), wing ($P = 0.041$), and drumstick ($P = 0.045$) compared to the control group. Dressing percentage showed a numerical improvement in the supplemented groups but was not statistically significant ($P = 0.083$).

Table 3 Effects of Glutamine Supplementation on Carcass carcass characteristics of heat stressed native crossbred chickens

Carcass qualities (12 weeks)	Control	L-Glu 1%	L-Glu 2%	SEM[1] (pool)	*P-value (<0.05)*
Live weight (g)	1,485.70	1,488.60	1,487.75	4.80	0.969
Carcass Yield (%)	74.40	74.45	74.55	0.08	0.726
Neck (g)	49.50	49.55	49.40	0.07	0.639
Head (g)	34.40	34.85	35.00	0.20	0.846
Wing (g)	142.50[a]	146.05[b]	145.90[b]	0.55	*0.008*
Breast (g)	397.80[a]	407.75[b]	408.65[b]	2.31	*0.013*
Drumstick (g)	170.45[a]	173.35[b]	174.10[b]	0.59	*0.026*
Frame (g)	309.20	308.95	308.25	0.19	0.789
Liver (g)	13.80[b]	12.80[a]	12.90[a]	0.17	*0.023*
Spleen (g)	2.50	2.51	2.45	0.07	0.938
Kidney (g)	4.15	4.25	4.30	0.10	0.833
Heart (g)	4.90	4.92	5.10	0.11	0.716

[1]SEM = pooled standard error of the mean, L-Glu = L-Glutamine
[a, b] = The means with different superscripts within the same row are significantly different at a statistical level (P<0.05).

Meat Composition

Proximate composition of breast meat is presented in Table 4. The protein content was significantly higher in both GLN-1% and GLN-2% groups (P = 0.032), while moisture, fat, and ash contents did not differ significantly among treatments (P > 0.05).

Table 4 Effects of Glutamine Supplementation on Meat Quality of heat stressed native crossbred chickens.

Meat qualities (12 weeks)	Control	L-Glu 1%	L-Glu 2%	SEM[1] (pool)	*P-value (<0.05)*
Shear force (N)					
Breast	2.47	2.52	2.58	0.07	0.847
Drumstick	4.54	4.31	4.58	0.15	0.855
Protein (%)					
Breast	22.45[a]	24.95[b]	24.15[b]	0.27	*0.013*
Drumstick	22.25[a]	23.55[b]	23.45[b]	0.18	*0.003*
Fat (%)					
Breast	8.75	8.25	8.70	0.22	0.613
Drumstick	9.25	9.85	9.40	0.28	0.659
Color					
L* (lightness)	40.29	39.88	39.83	0.18	0.543
a* (redness)	1.52	1.51	1.57	0.06	0.909
b* (yellowness)	2.01	1.96	1.94	0.11	0.958

[1]SEM = pooled standard error of the mean, L-Glu = L-Glutamine
[a, b] = The means with different superscripts within the same row are significantly different at a statistical level (P<0.05).

Oxidative Stress Indicators

The oxidative stress markers measured in plasma are summarized in Table 5. Birds supplemented with glutamine had significantly lower malondialdehyde (MDA) levels (P = 0.016) and increased concentrations of glutathione (GSH) (P = 0.014) and glutathione peroxidase (GPx) activity (P = 0.009). Cortisol concentrations were not significantly affected by dietary treatment (P > 0.05).

Table 5 Effects of Glutamine Supplementation on Oxidative Stress Indicators in heat- stressed native crossbred chickens.

Oxidative stress indicators	Control	L-Glu 1%	L-Glu 2%	SEM[1] (pool)	*P-value (<0.05)*
Malondialdehyde (MDA); µg/ml					
4 weeks	17.58[b]	13.66[a]	13.93[a]	0.57	*0.003*
8 weeks	17.28[b]	14.16[a]	13.73[a]	0.62	*0.030*
12 weeks	17.56[b]	14.26[a]	13.53[a]	0.61	*0.016*
Glutathione (GSH); µg/ml					
4 weeks	33.71[a]	49.30[c]	45.62[b]	2.46	*0.020*
8 weeks	31.64[a]	47.70[b]	47.10[b]	3.00	*0.041*
12 weeks	30.77[a]	48.15[c]	45.72[b]	2.75	*0.014*
Glutathione disulfide (GSSG); µmol/l					
4 weeks	12.18	11.48	10.77	0.51	0.551
8 weeks	12.73	11.10	11.47	0.57	0.260
12 weeks	12.37	11.40	11.71	0.46	0.691
Glutathione peroxidase (GPx); U/ml					
4 weeks	5.44[a]	6.62[b]	6.52[b]	0.21	*0.028*
8 weeks	5.32[a]	6.68[c]	6.07[b]	0.22	*0.032*
12 weeks	5.11[a]	6.56[b]	6.63[b]	0.24	*0.009*
Cortisol; ng/ml					
4 weeks	1.47	1.15	1.44	0.12	0.518
8 weeks	1.51	1.37	1.54	0.10	0.795
12 weeks	1.35	1.37	1.18	0.10	0.722

[1]SEM = pooled standard error of the mean, L-Glu = L-Glutamine
[a, b] = The means with different superscripts within the same row are significantly different at a statistical level (P<0.05).

DISCUSSION

The present study provides evidence that glutamine supplementation can effectively enhance the growth performance, carcass traits, and antioxidant status of native crossbred chickens raised under heat stress conditions. These findings are consistent with previous studies that have identified glutamine as a functional amino acid, especially in stressful physiological states (Yi et al., 2005; Wu, 2010; Kim et al., 2021). Under heat stress, birds experience reduced feed intake, impaired nutrient absorption, and increased production of reactive oxygen species (ROS), leading to oxidative damage and compromised productivity (Sahin et al., 2001). In our study, glutamine-supplemented birds, particularly at the 1% level, showed improved final body weight, average daily gain, and feed conversion ratio, despite no significant change in feed intake. These improvements may be attributed to glutamine's role in maintaining intestinal integrity, thereby enhancing nutrient absorption and overall feed efficiency (Newsholme, 2001; Li et al., 2007). Moreover, mortality rates were significantly reduced in the supplemented groups, especially with 2% glutamine. This suggests that glutamine also supports systemic physiological resilience, potentially through immune system modulation and intestinal barrier protection, as previously reported by Ncho et al. (2023). In terms of carcass traits, the observed improvements in breast, drumstick, and wing weights align with the enhancement in overall growth performance. While dressing percentage was not significantly different, the numerical increase in supplemented groups reflects positive production outcomes. The increase in protein content of breast meat without significant changes in moisture, fat, or ash, indicates better protein accretion, possibly due to improved amino acid utilization and muscle protein synthesis, as also noted by Yi et al. (2005).

The oxidative stress biomarkers provide further insight. The significant reduction in MDA levels and increase in GSH and GPx activity confirm that glutamine enhances the antioxidant defense system, helping to neutralize ROS and reduce lipid peroxidation. These results are in agreement with previous research in broilers under heat stress (Sahin et al., 2001; Wu, 2010). Compared with other non-essential amino acids such as glutamate, glycine, or proline, glutamine has a distinct functional role during physiological stress. While glutamate and glycine contribute to energy metabolism and neurotransmission, glutamine uniquely serves as both a nitrogen donor and a precursor for nucleotide synthesis, which is critical for rapidly dividing enterocytes (Wu, 2010; Chalvon-Demersay et al., 2021). Under heat stress, endogenous synthesis of non-essential amino acids may be insufficient to meet elevated metabolic demands. Glutamine becomes conditionally essential because of its role in maintaining intestinal barrier integrity, fueling immune cells, and enhancing antioxidant responses (Newsholme, 2001). Unlike other non-essential amino acids, glutamine directly supports the production of glutathione, a key intracellular antioxidant, thereby exerting both metabolic and protective effects under oxidative conditions. This multifaceted function gives glutamine a unique advantage in supporting poultry performance under heat stress when compared to other non-essential amino acids.

Interestingly, while glutamine supplementation at 2% showed antioxidant benefits, FCR was most favorable in chickens supplemented at 1%, suggesting that higher inclusion may not proportionally enhance growth performance, possibly due to a metabolic ceiling or energetic cost of processing excess glutamine (Li et al., 2007). Overall, these findings suggest that dietary glutamine supplementation at 1% is the optimal level, offering a balance between improved performance, carcass quality, oxidative stress reduction, and feed efficiency. These outcomes are particularly relevant to semi-intensive poultry systems in tropical environments, where birds are more vulnerable to ambient heat and production efficiency is often compromised.

CONCLUSIONS

In conclusion, dietary glutamine supplementation, particularly at 1% and 2% inclusion levels, improved growth performance, carcass traits, and antioxidant status in native crossbred chickens under heat stress conditions. While the 2% glutamine group exhibited the highest final body weight and the lowest feed conversion ratio, the 1% level demonstrated a more favorable balance between performance outcomes and feed cost per gain. Therefore, supplementation at 1% appears to be the optimal economic inclusion level under the conditions tested in this study. Glutamine supplementation at the 1% level, significantly enhances growth performance, carcass traits, and oxidative stress responses in native crossbred chickens reared under heat stress conditions. Glutamine improved body weight gain, feed efficiency, breast muscle development, and reduced lipid peroxidation, while boosting antioxidant enzyme activity. These improvements occurred without any adverse effects on feed intake or physiological stress indicators such as cortisol. Importantly, the 1% glutamine level provided the best balance of performance gains and feed efficiency, suggesting it is the optimal inclusion rate for native crossbred chickens raised in open-sided housing during hot seasons. These findings provide a practical nutritional strategy to mitigate heat stress in poultry production, especially in tropical and subtropical environments where climate control is limited. Further studies are warranted to investigate long-term effects, economic returns, and potential interactions with other functional nutrients or phytogenic compounds under commercial farm conditions.

ACKNOWLEDGEMENTS

This research was financially supported by the National Research Council of Thailand, Thailand. The authors express their sincere gratitude to the staff at the Poultry Research Unit, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, for their technical support and assistance during the trial. We also thank the students and laboratory technicians who participated in feed formulation, data collection, and sample analysis.

AUTHOR CONTRIBUTIONS

Montri Punyatong: investigation, methodology, formal analysis, writing original draft, validation, conceptualization, writing review and editing;
Wanaporn Tapingkae; Orranee Srinual; Chompunut Lumsangkul: research project participants, investigation, methodology, formal analysis. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article;

Montri Punyatong, Wanaporn Thapingkae, Orranee Srinual and Chompunut Lamsaengkul. Effects of glutamine supplementation on performance, carcass traits, and oxidative stress markers in native crossbred chickens under heat stress Conditions. Veterinary Integrative Sciences. 2026; 24(1): e2026001-1-9.