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The effects of dietary glutamine supplementation on growth performance and intestinal morphology of broiler chickens reared under hot conditions

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Abstract: Dietary supplementation effects of 0%, 0.25%, 0.5%, and 1% glutamine were investigated on the performance and development of the gastrointestinal tract in broilers reared under hot conditions. No significant differences were observed between the treatments for feed intake and feed conversion ratio during the starter period, grower period, or entire experiment ($P > 0.05$). During the grower period, only 0.5% glutamine-fed birds had higher body weight gain than others ($P < 0.05$). For the entire period, body weight gain of 0.5% glutamine-fed birds was greater than that of 0.00% and 0.25% glutamine-fed ones ($P < 0.05$). Furthermore, 0.5% glutamine-fed birds showed longer villus height than other birds ($P < 0.05$). In the jejunum, both 0.5% and 1% glutamine consumption caused longer villus height as compared to 0.00% and 0.25% glutamine ($P < 0.05$). Villus height of 0.25% glutamine-fed birds was higher than that of 0.00% glutamine-fed ones ($P < 0.05$). None of the ileum morphological parameters were affected by glutamine supplementation ($P > 0.05$). In conclusion, supplementation of 0.5% glutamine improved the intestinal morphology and body weight gain of broilers under heat stress. Higher dietary glutamine (1%) increased the villus height in the jejunum but did not change the performance.

Key words: Broiler chickens, glutamine, heat stress condition, intestinal morphology, performance

1. Introduction

Stress can have a profound effect on overall physiology, animal health, and productivity. The gastrointestinal tract is particularly responsive to stressors, which can cause a variety of changes including alteration of the normal protective microbiota (1) and decreased integrity of the intestinal epithelium (2). The nutritional stressors have deleterious effects on the absorptive epithelium of the intestine, resulting in reduction of villus height and crypt depth (3). Less is known about the effect of high environmental temperature on intestinal morphology. High environmental temperature decreases feed consumption, live weight, and feed efficiency in broiler chickens (4). Heat stress (HS) also significantly decreases the broilers' jejunal villus height (5). It causes less crypt depth, mucous area, and villus height of the duodenum and lowers the intestinal length in broiler chickens (6). Furthermore, reduced gut motility (7), changes in intestinal microflora (8), and a depression in the gastrointestinal blood flow have been noticed in broilers under HS conditions (9). Acute HS depresses enterocyte proliferation and affects the expression and activity of brush-border membrane enzymes in young birds (10). Thus, HS exposure may result in impaired digestibility of various essential amino acids. For example, decreased L-leucine transport has been reported across broilers' intestinal epithelium in in vitro HS studies (11). Accelerated replacement of enterocytes requires energy and proteins, which can negatively impact the growth and development of other tissues and organ systems (12).

Glutamine (Gln) is the principal metabolic fuel for small intestine enterocytes, lymphocytes, macrophages, and fibroblasts (13) and is considered as an essential amino acid in some species under inflammatory conditions of infection and injury (14). Many benefits of dietary Gln supplementation have been recognized in humans, rats, and pigs. In rats, Gln stimulated the gut mucosal proliferation (15). In another experiment on rats, dietary Gln (1.5%) maintained the gut integrity, which is important in preventing bacterial infections (16). It has even been shown to prevent intestinal hyperpermeability and bacterial translocation in mice during immunological challenge (17). In weanling pigs, Kitt et al. (18) showed improved feed efficiency by dietary addition of 1% Gln. Some beneficial effects of Gln have been recognized in broiler chickens, whereas no information is available on effects during HS. In an experiment on broiler chickens, Yi et al. (19) noticed ameliorated body weight gain and feed

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efficiency during the first week after hatching with Gln (1%) under normal temperature conditions.

To date, no research has been conducted on the use of dietary Gln in broiler chickens under HS, and so the present experiment evaluated the effect of Gln on growth performance and intestinal morphology of broilers reared under hot conditions.

2. Materials and methods

A total of 200 Ross 308 male broiler chicks of 1 day old were used in this study. The chicks were obtained from a local hatchery, weighed on arrival, and randomly allotted into 4 equal groups with 5 replicates each (10 chicks per replicate). The chicks were housed in a clean, well-ventilated room, previously disinfected with formalin. The room was provided with heaters to adjust the environmental temperature to 32 ± 1 °C and this temperature was maintained for 42 days. Relative humidity was 40%. Feeds and water were supplied ad libitum. Prophylactic measures against the most common infectious diseases were carried out. The chicks were vaccinated against Newcastle and Gumboro diseases as prescribed by a local veterinarian.

The treatment diets were formulated to meet the requirements of broiler chickens according to Ross requirement guidelines (Ross Company). The experimental diets were formulated to be isonitrogenous and isocaloric with 0.0%, 0.25%, 0.5%, and 1.0% Gln, which were identified as ZG (zero Gln), LG (low Gln), MG (medium Gln), and HG (high Gln), respectively. One percent finely ground, air-dried sand was used as an inert filler in the basal diet and different dietary levels of Gln were replaced by sand in the experimental diets (Table 1).

Gln with 98% purity (Serva Co., USA) was used in the current experiment. Average body weight gain (BWG) and feed intake (FI) were determined during the starter period (0 to 21 days of age), grower period (22 to 42 days of age), and whole experimental period (0 to 42 days of age). Moreover, the feed conversion ratio (FCR) was calculated for these periods. On days 21 and 42 of age, 1 bird from each replicate pen (5 per treatment) was killed by decapitation to collect the intestine samples. The intestine was removed and divided into 3 segments of duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducts to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction). The empty weight of these parts was determined separately. Approximately 5 cm of the middle portion of the duodenum, jejunum, and ileum was then cut, digesta was washed away using normal saline, and samples were fixed in 10% neutral buffered formalin. Following histological fixation, the tissues were processed through a standard alcohol dehydration-xylene sequence and embedded in paraffin. From each segment, 5 sections of 7 μm in thickness were made and they were stained with hematoxylin and eosin and periodic acid–Schiff stain. Morphometric analyses of digital photos of light microscopy were performed by means of Image J analysis software (20). Villus height (VH), villus width (VW), crypt depth (CD), muscular layer thickness (MLT), and goblet cells number (GCN) were determined and the VH to CD ratio (VCR) was calculated.

At the end of the experiment, 1 bird from each replicate was randomly selected and sacrificed. The weight and length of the different intestinal segments (duodenum, jejunum, and ileum) and their relative lengths (intestinal segment lengths/small intestine length × 100) and weights (organ weight/live body weight × 100) were calculated afterwards. All the data were submitted to the GLM procedure of the SAS program (21) based on a completely randomized design, with 4 treatments and 5 replicates each. Duncan's multiple range test at the level of $P < 0.05$ was used to denote statistical significance.

3. Results

3.1. Performance

Dietary supplementation effects of Gln on BWG, FI, and FCR during the starter, grower, and whole experimental periods are shown in Table 2. No Gln effect was detected for FI and FCR during the starter, grower, or whole experimental periods ($P > 0.05$). Similarly, no significant difference was determined between the treatments for BWG during the starter period ($P > 0.05$). During the grower period and the whole period, BWG was affected by Gln ($P < 0.05$). The BWG of MG-fed birds was greater than that of other birds during the grower period ($P < 0.05$), but there was no significant differences between other treatments. For the whole experiment, the BWG of MG-fed birds was higher than that of ZG- and LG-fed birds ($P < 0.05$). No significant difference was noted between HG birds and other treatments for BWG during the whole period ($P > 0.05$).

3.2. Intestinal morphology

The effects of dietary Gln on proportional intestinal part lengths and weights are shown in Table 3. The results of our experiment revealed that Gln supplementation had no effect on proportional intestinal part (duodenum, jejunum, and ileum) lengths (relative to intestine length × 100) and weights (relative to live body weight × 100) on days 21 and 42 of age ($P > 0.05$).

The intestinal morphology of broilers on day 21 of age is indicated in Table 4. In the duodenum, MG-fed birds had longer VHs than other birds ($P < 0.05$). Gln consumption had no effects on VW, CD, MLT, GCN, or VH to CD ratio ($P > 0.05$). In the jejunum, both the MG and HG birds had a higher VH than LG and ZG birds ($P < 0.05$). Furthermore, the VH of LG birds was higher than

Table 1. Ingredient composition and chemical analysis of the experimental diets (fresh basis).

	Starter diet (0–21 days)				Grower diet (22–42 days)			
	ZG	LG	MG	HG	ZG	LG	MG	HG
Ingredients (%)								
Corn	31.08	31.08	31.08	31.08	31.98	31.98	31.98	31.98
Wheat	20.00	20.00	20.00	20.00	25.00	25.00	25.00	25.00
Soybean oil	3.50	3.50	3.50	3.50	3.95	3.95	3.95	3.95
Soybean meal (44%) protein)	39.68	39.68	39.68	39.68	33.92	33.92	33.92	33.92
Dicalcium phosphate	2.10	2.10	2.10	2.10	2.15	2.15	2.15	2.15
Calcium carbonate	1.10	1.10	1.10	1.10	0.86	0.86	0.86	0.86
DL-methionine	0.38	0.38	0.38	0.38	0.08	0.08	0.08	0.08
L-Lysine	0.29	0.29	0.29	0.29	0.22	0.22	0.22	0.22
Vitamin and mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.37	0.37	0.37	0.37	0.34	0.34	0.34	0.34
Sand	1.00	0.75	0.50	0.00	1.00	0.75	0.50	0.00
L-Glutamine	0.00	0.25	0.50	1.00	0.00	0.25	0.50	1.00
Total	100	100	100	100	100	100	100	100
Calculated analysis								
Metabolizable energy (MJ/kg) (kcal/g)	2.85	2.85	2.85	2.85	2.95	2.95	2.95	2.95
DM (%)	85.16	85.16	85.16	85.16	85.43	85.43	85.43	85.43
CP (%)	22.01	22.01	22.01	22.01	20.00	20.00	20.00	20.00
Calcium (%)	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Sodium (%)	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15
Arginine (%)	1.54	1.54	1.54	1.54	1.38	1.38	1.38	1.38
Methionine + cystine (%)	1.07	1.07	1.07	1.07	0.73	0.73	0.73	0.73
Tryptophan (%)	2.13	2.13	2.13	2.13	1.94	1.94	1.94	1.94
Glycine (%)	0.97	0.97	0.97	0.97	0.89	0.89	0.89	0.89
Serine (%)	1.14	1.14	1.14	1.14	1.04	1.04	1.04	1.04
Glycine+ serine (%)	2.11	2.11	2.11	2.11	1.93	1.93	1.93	1.93
Histidine (%)	0.60	0.60	0.60	0.60	0.55	0.55	0.55	0.55
Isoleucine (%)	0.97	0.97	0.97	0.97	0.88	0.88	0.88	0.88
Leucine (%)	1.81	1.81	1.81	1.81	1.66	1.66	1.66	1.66
Lysine (%)	1.43	1.43	1.43	1.43	1.24	1.24	1.24	1.24
Methionine (%)	0.70	0.70	0.70	0.70	0.38	0.38	0.38	0.38
Cystine (%)	0.37	0.37	0.37	0.37	0.35	0.35	0.35	0.35
Phenylalanine (%)	2.13	2.13	2.13	2.13	1.04	1.04	1.04	1.04
Tyrosine (%)	0.98	0.98	0.98	0.98	0.89	0.89	0.89	0.89
Phenylalanine + Tyrosine (%)	2.13	2.13	2.13	2.13	1.94	1.94	1.94	1.94
Threonine (%)	0.85	0.85	0.85	0.85	0.77	0.77	0.77	0.77
Valine (%)	1.08	1.08	1.08	1.08	0.98	0.98	0.98	0.98

[1]Supplied per kilogram of diet: retinol, 9000 IU; cholecalciferol, 2000 IU; tocopherol, 18 IU; cyanocobalamin, 15 mg; riboflavin, 6.6 mg; pantothenate, 10 mg; niacin, 30 mg; choline, 500 mg; biotin, 0.1 mg; thiamine, 1.8 mg; pyridoxine, 3 mg; folic acid, 1 mg; menadione, 2 mg; ethoxyquin, 100 mg; zinc, 50 mg; manganese, 100 mg; copper, 10 mg; iron, 50 mg; iodine, 1 mg; selenium, 0.2 mg.
ZG = 0.0% glutamine, LG = 0.25% glutamine, MG = 0.5% glutamine, HG = 1% glutamine.

Table 2. The effects of L-glutamine on performance of broiler chickens reared under hot conditions during the starter (0 to 21 day of age), grower (22 to 42 days of age), and whole experimental (0 to 42 days of age) periods.

	Treatments	FI (g)	BWG (g)	FCR
Starter	ZG	1066.08	680.50	1.56
	LG	1038.91	661.40	1.57
	MG	1071.62	694.50	1.54
	HG	1052.39	662.50	1.59
	Pooled SEM	7.38	7.29	0.02
	P-value	0.39	0.36	0.86
Grower	ZG	2879.45	1285.13^{b}	2.21
	LG	2918.04	1339.64^{b}	2.17
	MG	3047.62	1478.05^{a}	2.06
	HG	3004.74	1354.13^{b}	2.22
	Pooled SEM	31.27	23.92	0.03
	P-value	0.21	0.02	0.19
Whole the experimental period	ZG	3945.50	1975.25^{b}	1.98
	LG	3957.00	2001.04^{b}	1.98
	MG	4105.80	2179.41^{a}	1.88
	HG	4061.70	2053.05^{ab}	1.97
	Pooled SEM	36.52	27.89	0.02
	P-value	0.39	0.04	0.33

$^{a, b}$: Mean values with different superscripts within each column and period are significantly different ($P < 0.05$).
BWG = Body weight gain, FI = feed intake FCR = feed conversion ratio, ZG = 0.0% glutamine, LG = 0.25% glutamine, MG = 0.5% glutamine, HG = 1% glutamine.

Table 3. Effect of dietary L-glutamine supplementation on proportional intestinal part (duodenum, jejunum, and ileum) lengths (relative to intestine length × 100) and weights (relative to live body weight × 100) in broiler chickens on day 21 of age and reared under hot conditions.

	Treatments	ZG	LG	MG	HG	Pooled SEM	P
Duodenum	Weight	1.34	1.52	1.36	1.51	0.05	0.39
	Length	22.01	20.05	21.02	21.22	0.82	0.89
Jejunum	Weight	2.95	2.70	3.18	2.74	0.09	0.17
	Length	44.99	43.88	43.12	43.34	0.56	0.68
Ileum	Weight	2.13	1.46	1.77	1.57	0.12	0.18
	Length	32.99	36.07	35.85	35.44	0.58	0.22

ZG = 0.0% glutamine, LG =0.25%, glutamine, MG = 0.5% glutamine, HG = 1% glutamine.

Table 4. Effect of dietary L-glutamine supplementation on duodenum, jejunum, and ileum morphology in broiler chickens on day 21 of age and reared under hot conditions.

	Treatments	Glutamine supplementation (%)					
		ZG	LG	MG	HG	Pooled SEM	P-value
Duodenum	VH (μm)	1394.73[b]	1410.18[b]	1449.45[a]	1390.97[b]	6.84	0.0005
	VW (μm)	142.44	140.77	140.35	145.78	1.29	0.47
	CD (μm)	149.96	143.27	149.54	146.62	1.39	0.32
	MLT (μm)	190.48	185.46	182.12	187.97	1.38	0.17
	GCN (mm^2)	7162.86	7125.27	7176.23	7108.56	13.37	0.25
	VH to CD ratio	9.31	9.84	9.71	9.49	0.06	0.21
Jejunum	VH (μm)	1109.02[c]	1145.36[b]	1182.11[a]	1187.54[a]	9.51	0.0009
	VW (μm)	81.87	81.45	81.45	82.71	1.01	0.97
	CD (μm)	96.49	99.83	105.26	103.17	1.33	0.08
	MLT (μm)	143.69[a]	133.25[b]	142.87[a]	141.19[a]	1.34	0.006
	GCN (mm^2)	8166.20	8180.40	8276.47	8297.36	23.07	0.08
	VH to CD ratio	11.52	11.49	11.23	11.52	0.12	0.84
Ileum	VH (μm)	489.55	489.55	508.35	529.65	6.90	0.11
	VW (μm)	75.61	76.44	76.44	74.77	1.10	0.95
	CD (μm)	69.34	69.76	68.09	66.83	1.00	0.77
	MLT (μm)	130.74	127.82	131.16	134.08	1.27	0.42
	GCN (mm^2)	10,080.14	10,077.63	10,082.64	10,037.53	19.28	0.85
	VH to CD ratio	7.06	7.05	7.47	7.96	0.16	0.13

[a–c]: Mean values with different superscripts within a row are significantly different ($P < 0.05$).
VH = Villus height, VW = villus wide, CD = crypt depth, MLT= muscular layer thickness, GCN = goblet cell number, VH to CD ratio = villus height to crypt depth ratio, ZG = 0.0% glutamine, LG = 0.25%, glutamine, MG = 0.5% glutamine, HG = 1% glutamine.

that of ZG birds ($P < 0.05$). No significant differences were seen between treatments for other variables ($P > 0.05$). In the ileum, VH, VW, CD, MLT, GCN, and VH to CD ratio were not affected by Gln in diet ($P > 0.05$).

4. Discussion

Many investigators presented the negative effects of HS on gut morphology. Mitchell and Carlisle (5) indicated the lower jejunal villus height in broiler chickens under HS. Marchini et al. (6) showed that HS reduces the crypt depth, mucous area, villus height of the duodenum, and even intestinal length in broiler chickens. Hence, the present experiment investigated the possible positive effects of Gln on intestinal development and performance of broiler chickens under HS. As expected, consumption of 0.5% Gln in birds under HS conditions resulted in longer VHs in the duodenum and jejunum. It has been stated that addition of Gln to broiler diets positively affects the gastrointestinal tract and could increase the villi length in different segments of the small intestine under normal temperature conditions (22–24). In an experiment on turkey poults, Gln supplementation increased the intestinal villus height and decreased crypt depth (25). Fischer da Silva et al. (23) observed an increased number of intestinal villi with 1% Gln in feed-restricted broiler chickens. Soltan (22) indicated similar results with Gln in broiler chickens vaccinated against Newcastle disease. In another consistent experiment, Murakami et al. (24) reported longer villi in the jejunum on days 14 and 41 after hatching in broilers fed diets including 1% Gln and vitamin E. The positive effect of Gln on VH is related to its nutritive importance for villus growth. Gln is an important

amino acid for utilization as an energy source for the development of the mucosa and stimulates intestinal cell proliferation, thus increasing the absorptive surface of the gastrointestinal mucosa and the utilization of nutrients (26).

Villus height is positively related to villus surface area (5). The expansion of surface area that occurs with villus growth has been used to explain the increased absorptive capacity, whereas decreased villus height lowers the absorptive capability of the small intestine (3). Hence, a higher villus height increases the intestinal surface area and consequently nutrient absorption (22), and results in better performance. We found better BWG for 0.5% Gln-fed birds, which may be related to their longer VHs at day 21 of age and therefore improved nutrient utilization. There are some consistent studies regarding the positive effects of dietary Gln on performance of broiler chickens and especially BWG. Although Murakami et al. (24) and Maiorka et al. (27) found no effect of Gln on BWG (days 1–41) of broiler chickens, in agreement with our results, Bartell and Batal (28) observed higher BWG during the third week of age with 1% Gln. Soltan (22) reported improved daily BWG in broilers fed 1% Gln under normal temperature conditions. In the same temperature conditions, Devi Priya et al. (29) showed that 0.5% Gln administration increased the body weight of broilers during the entire experimental period.

Even though 1% Gln caused the same villus lengths as 0.5% Gln supplementation, it did not increase the BWG. The first possible reason for this negative effect on BWG is the role of Gln in ammoniagenesis, which increases the energy requirement for excretion of uric acids. The Gln acts as a precursor for ammoniagenesis in the gut and kidneys (30). In birds, ammonia is excreted in feces in the form of uric acid and is involved in uric acid synthesis (22). The second reason is possibly the production of high ammonium ions in 1% Gln-fed birds. The Gln is converted to α-ketoglutarate and thus generates ammonium ions (NH_4). Although excretion of ammonium ions helps buffer metabolic acidosis in normal temperatures (31), it negatively affects the blood acid–base balance during HS-induced alkalosis and hence does not improve BWG. It is thought that panting during HS results in respiratory alkalosis and higher blood pH consequently. Thus, high dietary inclusion of Gln (1%) in broiler chickens under HS increases the ammonium excretion and exacerbates the HS-induced alkalosis, and, as consequence, it does not improve the performance as compared to 0.5% Gln.

In conclusion, the results of this study suggest that 0.5% Gln in the diet improves the intestinal morphology and consequently the BWG of broiler chicks under hot conditions. Although a higher level of dietary Gln (1%) increased the villus height in the jejunum, it did not change the performance.

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