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Influence of Supplemental Glutamine on Nutrient Digestibility and Utilization, Small Intestinal Morphology and Gastrointestinal Tract and Immune Organ Developments of Broiler Chickens

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Abstract**—**This study was conducted to investigate the optimum levels of glutamine (Gln) supplementation in broiler diets. A total of 32 one-day-old male chicks with initial body weight 41.5 g were segregated into 4 groups (8 chicks per group) and subsequently distributed to individual cages. Feed and water were provided *ad libitum* for 21 days. Four dietary treatments were as follows: control and supplemented Gln at 1, 2 and 3%, respectively. The results found that the addition Gln had no negative effects on dry matter, organic matter, ash digestibility or nitrogen retention. Birds fed with 1% Gln had significantly higher villi wide and villi height : crypt depth ratio in duodenum than the control chicks and 2 and 3% Gln chicks. It is suggested that the addition of Gln at 1% indicated a beneficial effect on improving small intestinal morphology, in addition Gln may stimulate immune organ development of broiler chickens.

Keywords**—**broiler chicken, digestibility, gastrointestinal tract glutamine, glutamine

I. Introduction

DUE to the ban on the use of antibiotics as growth promoters to improve growth performance and to control diseases in poultry feed, there have been numerous problems leading to depressed growth performance and an increased the incidence of disease. The supplementation of glutamine (Gln) is an alternative feed additive that should be studied in broiler diets. Glutamine is the most prevalent amino acid in the bloodstream, accounting for 30-35% of the amino acid N in the plasma and in the free amino acid pool in the body [1].

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Numerous literatures reported that Gln is the principle metabolic fuel for small intestine enterocytes, lymphocytes, macrophages and fibroblasts and is considered an essential amino acid in some species under inflammatory conditions [2], [3]. Many benefits have been observed due to Gln supplementation in the diet of humans and rats, however, little research has been done with poultry. Therefore, this study was aimed to investigate the effect of Gln supplementation on nutrient digestibility and retention, small intestinal morphology and gastrointestinal tract and immune organ developments of broiler chickens.

II. Materials and Methods

A total of 32 one-day-old male chicks with initial body weight 41.5 g were segregated into 4 groups (8 chicks per group) and subsequently distributed to individual cages. Feed and water were provided *ad libitum* for 21 days. Four dietary treatments were as follows: control and supplemented Gln at 1, 2 and 3%, respectively (Table 1). All nutrients were formulated to meet or exceed the minimum NRC [4] requirements for broiler chickens.

Excreta were collected on 18 to 21 days of age. The excreta were sprayed with 5% HCl and dried at 55^{o}C. Dried excreta were stored at -20^{o}C for later analyses. DM, organic matter and N in the diets and excreta were measured to assess their digestibilities and retention according to standard methods [5]. At the end of the experiment, the birds were weighed and killed by cervical dislocation and then the abdominal cavity was open. The thymus and spleen were removed and weighed. For intestinal weight measurements, the small intestine was removed and divided into 3 segments: duodenum, jejunum and ileum. The ileum was flushed with 10 to 20 ml of deionized water and the empty weight was recorded. While duodenum and jejunum were flushed with 20 ml saline solution and the empty weigh was recorded. Organ weights were expressed on a weight relative to live body weight (g/100g of BW). For morphologic analysis, approximately 5 cm of the middle portion of the duodenum and jejunum was excised and fixed with 10% formalin. The cross sections of 70% ethanol-preserved segments for each duodenal and jejuna sample were

TABLE I
COMPOSITION OF THE EXPERIMENTAL DIETS (AS FED BASIS)

Item	Control	Glutamine levels (%) 1	2	3
Ingredients, %				
Corn	48.64	47.06	47.06	46.17
Soybean meal	28.65	28.72	28.72	28.84
Fish meal	9.00	9.00	9.00	9.00
Rice bran	5.00	5.00	5.00	5.00
Cassava starch	3.00	2.00	1.00	0.00
Soybean oil	3.10	3.86	4.60	5.36
Salt	0.25	0.25	0.25	0.25
DL-Methionine	0.26	0.27	0.27	0.28
Glutamine	0.00	1.00	2.00	3.00
Calcium carbonate	0.06	0.60	0.60	0.60
Dicalcium phosphate	1.00	1.00	1.00	1.00
Premix[1]	0.50	0.50	0.50	0.50
Calculated composition, %				
AME, kcal/kg	3102	3102	3102	3102
Met + Cys	0.90	0.90	0.90	0.90
Lys	1.20	1.20	1.20	1.20
Ca	1.02	1.02	1.02	1.02
Available P	0.62	0.62	0.62	0.62
Analyzed composition, %				
DM	92.06	92.29	92.22	92.51
CP	21.43	22.36	22.37	24.13
CF	2.91	2.80	2.84	2.90
EE	6.39	7.21	7.31	8.75

[1]Premix (0.5%) provided the following per kilogram of diet: vitamin A, 15,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; vitamin K_3, 5 mg; vitamin B_1, 2.5 mg; vitamin B_2, 7 mg; vitamin B_6, 4.5 mg; vitamin B_{12}, 25 µg; pantothenic acid, 35 mg; folic acid, 0.5 mg; biotin, 25 µg; nicotinic acid, 35 mg; choline chloride, 250 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; Cu, 1.6 mg; I, 0.4 mg; Se, 0.15 mg.

then prepared for staining with hematoxylin and eosin using standard paraffin embedding procedures [6].

Data were analyzed by ANOVA and using SPSS version 13.0 [7]. Significant differences among treatment were assessed by Duncan's new multiple range-test.

III. Results

Nutrient digestibility and retention of broiler chickens fed with Gln is presented in Table 2. The results found that Gln had no negative effects on dry matter, organic matter and ash digestibility and N retention. Although these values were numerically decreased with increasing Gln in diets but there were not statistically differences (P>0.05). The percentages of dry matter (DM), ash and organic matter digestibility and N retention were 74.01, 72.60, 72.55 and 69.83%; 38.88, 38.90, 38.52 and 38.32; 77.80, 75.92, 72.55 and 67.52, 66.70, 66.07 and 64.40% in broilers fed control and supplemented with 1, 2 and 3% Gln, respectively.

TABLE II
EFFECT OF GLUTAMINE SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY AND RETENTION[1]

	Control	Glutamine levels (%) 1	2	3
DM (%)	74.01	72.60	72.55	68.83
Ash (%)	38.88	38.90	38.52	38.32
OM (%)	77.80	75.92	72.55	68.83
N retention (%)	67.52	66.70	66.07	64.40

[1] Values for each parameter represent mean values of 8 observations

The effect of Gln on digestive and immune organ relative weights of broilers is summarized in Table 3. The data revealed that Gln supplementation had no effect on bursa, small intestine and cecum relative weights of broilers (P>0.05). While the spleen relative weight was significantly heavier with the addition of 3% Gln compared with the control and 1 to 2% Gln diets. The weights of spleen (g/100g BW) were 0.12, 0.09, 0.11 and 1.99 in broilers fed with control and supplemented with 1, 2 and 3% Gln, respectively.

TABLE III
EFFECT OF GLUTAMINE SUPPLEMENTATION ON DIGESTIVE AND IMMUNE ORGAIN WEIGHTS OF BROILERS (G/100G BW)[1]

	Control	Glutamine levels (%) 1	2	3	SEM
Spleen	0.12[b]	0.09[b]	0.11[b]	1.99[a]	0.0553
Bursa	0.24	0.21	0.28	0.32	0.0541
Duodenum	1.16	0.96	0.88	0.97	0.1131
Jejunum	1.69	1.50	1.48	1.58	0.1422
Ileum	1.33	1.27	1.24	1.22	0.0722
Ceca	1.24	0.73	0.81	0.69	0.1855

[a, b] Means with different superscripts in a row are significantly different (P<0.05)
[1] Values for each parameter represent mean values of 2 observations

Glutamine supplementation in diets did not affect the villi height and crypt dept both in duodenum and jejunum of broilers (P>0.05) (Table 4). The values of villi height in the duodenum and jejunum were 331, 449, 248, 180 µm and 257, 552, 333 and 320 µm in broilers fed control and supplemented with 1, 2 and 3% Gln, respectively. The values of crypt dept in duodenum and jejunum were 125, 132, 124 and 102 µm, and 93, 107, 97 and 98 µm in broilers fed control and supplemented with 1, 2 and 3% Gln, respectively. While the birds fed diets supplemented with 1% Gln had significantly higher villi width in the duodenum than control and 2 to 3% Gln birds. The values of villi width in the duodenum were 86, 92, 68 and 51 µm in broilers fed control and supplemented with 1, 2 and 3% Gln respectively, in which the addition of 2 and 3% Gln resulted in decreased villi width (P<0.05). However, the addition of Gln had no negative effect on decreased villi width in jejunum. The ratio of villi height : crypt dept in duodenum were increased according with the villi width. These ratios increased in 1% Gln but decreased in 2 and 3% Gln when compared to control birds (P<0.05). However, the ratios of villi height : crypt dept were not significantly in jejunum. The values of villi height : crypt dept ratio in the duodenum and jejunum were 2.7, 3.4, 2.0, 1.8 and 2.7, 5.0, 3.4, 3.0 in broilers fed control and supplemented with 1, 2 and 3% Gln, respectively.

IV. Discussion

This study found that the addition of Gln at 1% showed the highest efficacy without any negative effects on dry matter, organic matter, ash digestibility or nitrogen retention. Birds fed with 1% Gln had significantly higher villi width and villi height : crypt depth ratio in duodenum than the control chicks. This finding according to many previous studies indicated the same effect of Gln supplementation in broiler chick diets on increased villi height in small intestine [8]-[10]. In addition,

TABLE IV
EFFECT OF GLUTAMINE SUPPLEMENTATION ON SMALL INTESTINAL MORPHOLOGY[1,2]

Item	Control	Glutamine levels (%) 1	2	3	SEM
Villi height (µm)					
Duodenum	331	449	248	180	63.5418
Jejunum	257	552	333	320	99.21
Crypt depth (µm)					
Duodenum	125	132	124	102	17.9529
Jejunum	93	107	97	98	16.6100
Villi wide (µm)					
Duodenum	86[b]	92[a]	68[c]	51[d]	1.5116
Jejunum	99	102	88	81	11.3400
Villi height : Crypt depth					
Duodenum	2.7[b]	3.4[a]	2.0[c]	1.8[c]	0.0075
Jejunum	2.7	5.0	3.4	3.0	0.5408

[a-d] Means with different superscripts in a row are significantly different ($P<0.05$)
[1] Values for each parameter represent mean values of 2 observations
[2] Small intestine sample of each treatment was taken from chickens aged 21 days

Bartell and Batal [11] also reported a beneficial effect of Gln on increased intestinal villi height when Gln was added to diets at 1 and 4%. Because Gln is an amino acid important for utilization as energy source for the development of mucosa and stimulate intestinal cell proliferation, thus it increasing the absorptive surface of gastrointestinal mucosa and the utilization of nutrients [11]. However, broilers fed diets supplemented with 2 and 3% Gln had a significantly lower intestinal villi width than the broilers fed control and 1% Gln diets. This phenomenon is still not clear. Soltan [10] investigated the effect of Gln supplementation at 0.5, 1.0, 1.5 and 2% in broiler diets and concluded that the addition of 1% Gln can be improved growth performance and may stimulate the development of gastrointestinal tract and immune response, while higher level had negative effects. Normally, if the intestinal villi height can be increased early in the chick's life, then the chick may be able to utilize nutrients more efficiently earlier in life and thus have improved growth performance [11]. In addition, Nitsan et al. [12] also stated that birds with a faster growth rate have a high capability to secrete high levels of enzymes, implying that initial growth is only limited by the early development of the digestive organs, therefore, reducing the time for development of digestive organs, growth improvements could be achieved. Even though the birds fed with 1% Gln has increased villi width in comparison with the control or 2 and 3% Gln, nutrient digestibility and retention were not significantly different among treatments. This may be due to the fact that all dietary nutrients are balance and are sufficient for broiler requirements, or it could also suggest that increased villi width does not necessarily lead to increased nutrient utilization.

Immune tissue development is the basis of immune functionality. The supplementation of Gln at levels of 3% significantly promoted the growth of the spleen but had no effect on the bursa weight. These findings are in agreement with the previous studies which reported the improvement of spleen and thymus weights in broiler chicks fed on diets supplemented with Gln [10], [11], [13]. Glutamine is also the precursor for the net synthesis of arginine, which has been shown to increase thymus and spleen size in mice [14] increase cytokine production and enhance lymphocyte proliferation [15].

Based on the above studies it is suggested that the addition of Gln 1% indicated the most advantageous on improving small intestine morphology. In addition Gln may stimulate immune organ development of broiler chickens. This finding may be useful for newborn chicks, since their system, especially digestive and immune system is not fully developed and the chicks are more susceptible to disease or likely to be negatively impacted by their environment.

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