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[1]Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh;
[2]Department of Animal Science, Chonbuk National University, Jeonju 561-756, Republic of Korea

Corresponding author:
Dr. Md. Rakibul Hassan
Bangladesh Livestock Research Institute
Savar, Dhaka-1341, Bangladesh
Email: mdrakibulhassan@gmail.com

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Research Article

Effect of dietary supplementation of Tryptophan, Arginine, and Glutamine on growth performance, gut morphology, immune response and meat quality of broiler chicken

MR Hassan[1]*, S Sultana[1], N Sultana[1]and KS Ryu[2]

Abstract

Two experiments were conducted to evaluate the supplemental effects of Arginine (Arg), Glutamine (Gln) and Tryptophan (Trp) on growth performance, gut morphology, immune response and meat quality of broilers. In experiment 1, 312 one-day-old Ross×Ross broiler chicks with similar body weight were randomly assigned to 4 dietary treatments (C, control having no amino acids; Arg, a diet with 0.5% L-Arg; Gln, a diet with 0.5% L-Gln and Trp, a diet with 0.5% L-Trp), each containing 6 replicate cages with 13 birds per cage. For further confirmation, experiment 2 considered similar treatments as of experiment 1. Body weight and feed intake were measured weekly. At 2nd and 6th weeks of age, 12 birds per treatment were killed in both experiments to measure gut morphology, serum IgG and IgA and meat quality of broilers. In Expt 1, results showed that dietary Arg, Gln and Trpsupplementation significantly increased ($P< 0.05$) weight gain and feed intake of broilers than those of control group. In experiment 2, a similar performance trend was obtained compared to Experiment 1. Circulating levels of IgG and IgA were increased with supplementation of AAs in the diet. In both experiments, the breast and drumstick muscles of the group of birds feed amino acids had a higher pH and redness (a*) values, but lower lightness (L*) and yellowness (b*) values of meat. Chicks fed diet with Arg, Gln and Trp had longer villi height and villi to crypt depth ratio, and shorter crypt depth as compared with the control ($P<0.05$). Thus the addition of Arg, Trp and Gln each at 0.5% in the diet of broiler chicken enhanced growth performance, immunity, gut morphology and meat quality characteristics of broiler.

Introduction

In recent years growth performance of modern commercial broiler strains has been continually improved with the advancement of genetic selection (Leeson, 2005) and such improvement are likely to affect the maintenance and growth needs for amino acids. Therefore, NRC (2005) recommendation of amino acid might not be optimal to support the maximal growth potential of the high yielding strains. This is however, optimum level of amino acid in the diet would increases weight gain, whereas decreasing the amino acid level causes reduced weight gain and FCR of broilers (Dozier et al., 2008). Therefore, an optimum amino acid concentration in broiler chicks is a strategy to maximize the performance and mitigate diet cost. Meanwhile, researcher's attention is now centered upon on a specific amino acid requirement of broiler chicks. In particular, glutamine, tryptophan and arginine are among the important 'bioactive amino acids', and participate in many important and diverse biochemical reactions associated with the normal physiology of the bird. L-tryptophan is an essential amino acid and plays a rate limiting role in protein and niacin biosynthesis. It also a precursor of serotonin and melatonin (Emadi et al., 2010), which control of circadian rhythms and is associated with blood pressure, body temperature, feed intake, growth and repair of tissues (Corzo et al., 2005). Consequently, glutamine may be a vehicle for nitrogen exchange between tissues, and may play an essential role in several important metabolic pathways (Smith, 1990; Jazideh et al., 2014). It also recognized as a crucial energy substrate in rapidly dividing cells, and may act on the humoral immune response (Bartell and Batal, 2007) and development of gastrointestinal tract of broiler chicks (Rahim et al., 2017; Newsholme et al., 2003). Therefore, Moghaddam and Ghamsari (2013) and Yi et al. (2005) observed that 1% glutamine in the diet would enhance the performance of broiler chicks Due to the lack of some enzymes in the urea cycle, broiler chickens are unable to biosynthesize arginine from ornithine; thus, this amino acid must be supplemented to meet their needs for protein synthesis and immune responses (Tayade et al., 2006). However, in modern broiler industry, the dietary supplementation of specific amino is increasing used to enhanced performance, but relatively little attention has been given to the meat quality and gut morphology of broiler chicks. Thus, there is an important issue to know the effects of dietary supplementation of amino acid on the performance and meat quality of broilers. Therefore, two experiments were conducted to clarify the effects of amino acids on the performance, gastrointestinal tract morphology, meat quality and immunity of broiler chickens.

Materials and methods

For each of the two experiments, 312, day-old chicks were weighed and divided equally into four dietary treatments with six replications. In both experiments, treatments were considered as follows: 1) control (corn-soybean meal based diet without Arginine, glutamine or Tryptophan supplementation); 2) a diet including 0.5% tryptophan (Trp) supplement from L-Trp; 3) a diet including 0.5% Arginine (Arg) supplement from L-Arg and 4) a diet including 0.5% glutamine (Gln) supplement from L-Gln. The chicks were housed in separate pen with a ventilation fan in the central arena that circulated fresh air inside the cages. The room temperature was maintained at 33˚Cfor the first week, and then reduced by 2-3˚C per week until it reached 22˚C, which was maintained until the end of the experiment. Mean relative humidity was maintained at 60-65% throughout the experiment. The broilers had free access to commercial pellet (From 1 to 7 d of age, a pre-starter pellet with 205 g CP and 3,000 kcal ME/kg was fed, followed by a starter ration with 185 g CP and 3,050 kcal ME/kg and grower ration with 175 g CP and 3,100 kcal ME/kg up to the end of the experiment) and water at all times.

Birds were individually weighed weekly to determine the mean population weight. Therefore, average weight gain was expressed on a bird basis with total pen weight divided by final bird count per pen for that period. Feed intake was expressed on a bird basis with total feed consumed per pen divided by final bird count per pen for that period. The FCR was also expressed as total feed consumed per pen divided by total pen weight. All management of chicks and experimental procedures were conducted in accordance with the commercial breeding company, Republic of Korea. The broilers had free access to corn and soybean meal based diet and water at all the time.

At the end of the experiment and 12 h after withdrawal of feed, 12 birds per treatment were sacrificed and breast and thigh muscles were removed from the carcass. The muscular pH values were determined using a digital pH meter and meat color (CIE, lightness L*, redness a* and yellowness b*) were measured using a Minolta colorimeter. At 2nd and 6th weeks of age, 12 birds per treatment were sacrificed and jejunum (Meckel's diverticulum), and ileum segments were taken and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4, 4°C) for 48 h, and paraffin sections (5 µm thick) were made. Sections were stained with hematoxylin and eosin. Eight cross-sections for each intestinal segment were prepared for each bird. Villus height and crypt depth were measured from 5 vertically oriented crypts and villi structures of each section by using image analysis software. To measure IgG and IgA, blood was collected and separated serum was stored at -70°C until analysis. Both IgG and IgA were measured using chicken ELISA quantitation kits (Bethyl Laboratories, Inc. Montgomery, TX, USA) as described by Perez-Carbajal et al. (2010). All data were analyzed by one-way analysis of variance using the GLM procedure in SAS (2002). Duncan's new multiple-range test was performed to identify differences (Steel and Torrie, 1980). The P-values of less than 0.05 were considered significant.

Results and discussion

Growth performance

In both experiments, significant improvements in weight gain and feed intake were observed when 0.5% of each of Gln, Trp and Arg were supplemented in the feed as compared with the control diet (Table 1). The present results also have shown that broiler chicks supplemented with 0.5% Trp significantly ($P<0.05$) increased growth performance and feed intake. However, no significant differences were observed in weight gain and feed intake among the Arg, Trp and Glu treatments. It might be due to the function of Trp as a precursor of serotonin (Denbow et al., 1993) and this serotonin formed in the brain and influences feed intake (Wang et al., 2012). In previous, Davila et al. (1987) mentioned that, Arg influences to the release of pituitary and pancreatic hormones and which regulates protein synthesis, thereby increasing feed intake and which lead to increase growth performance. In another experiment, Yi et al. (2005) evaluated the influence of Gln and reported better feed efficiency, weight gain and viability of broiler chicks which corresponds with the present findings.

Serum IgA and IgG

In experiment 2, dietary supplementation of Arg and Gln resulted in higher ($P>0.05$) levels of IgG, IgA than those of the birds reared under the control treatment (Table 2). This may indicate that the birds fed diets supplemented with 0.5% Gln, Trp or Arg had a better gut barrier function. This finding is supported by the previous studies done by Mathers and Cuff (2004) who reported that IgG levels increased in the birds fed diets supplemented with Gln. In another experiment, Bartell and Batal (2007) found that the birds fed diets supplemented with 1% Gln had significantly higher IgA concentrations in the serum which indicated better health and resistant to infection.

Carcass and meat qualities

Generally, pH value is a direct reflection of muscle acid content and affects color in meat and color is a major criterion to judge meat quality (Jakobsen and Bertelsen, 2000). In the present two consecutive experiments, carcass weight, breast and thigh meat weight were increased numerically by the AA supplemented groups compared to that of the control group (Table 3), but, no significant differences were observed among the Arg, Trp and Glu treatments. The breast and drumstick muscles from amino acid feeding groups had a higher pH and redness (a*) values, but lower lightness (L*) and yellowness (b*) values of meat (Table 3) might be due to supplementation of AA into the diet. Similarly, Denbow et al. (1993) reported that supplementation (0.5%) of dietary Trp may play a regulatory role of serotonin and this serotonin reduces the release of cathecholamines (cortisol, epinephrine, etc.) and thus increased pH and CIE (a*) value of meat. In another experiment, Dai et al. (2009) added 0.5 to 1% Gln in the broiler's diet and thus improve meat quality. Therefore, 0.5% dietary inoculation of Trp or Gln would be effective to improve meat quality of broiler chicks.

Gut morphology

If the intestinal villi height can be increased early in chick's life, then chicks may be able to utilize nutrients more efficiently and thus improved growth performance. In experiment 1, diets supplemented with 0.5% Arg, Trp and Gln preparation had the higher (P<0.05) villus height and villus height:crypt depth ratio and consequently the lower crypt depth in comparison to the control group (Table 3). The present results indicated that the birds fed diets supplemented with Arg had a longer villi height than the control diet. The longer villi height should increase surface area and consequently greater nutrient absorption by the Arg treatment and thus improved weight gain of broiler chicks. Bartell and Batal (2007) mentioned that broiler fed diets supplemented with Gln had significantly longer intestinal villi than the control corn-SBM diet. Recently, Rahim et al. (2017) concluded that dietary 0.5% Gln alone or along with 130% Arg improves the intestinal morphology and performance of broiler chicken and which corresponds with the present results. Therefore, chicks might be able to utilize nutrients more efficiently in the early stage under the amino acid treatments and thus improved growth performance (Nitsam et al., 1991).

Table 1: Effect of different amino acids on the performance of broiler chicks

Treatments	C	A	G	T	SEM	P value
Experiment 1						
Initial weight (g)	44.18	44.19	44.21	44.22	0.03	0.985
Final weight (g)	2231.71^{b}	2547.19^{a}	2399.98^{a}	2530.50^{a}	44.13	0.003
Weight gain (g)	2187.53^{b}	2503.75^{a}	2355.77^{a}	2486.28^{a}	44.07	0.003
Feed intake (g)	3688.62^{b}	4151.54^{a}	4023.20^{a}	4136.46^{a}	55.29	0.003
FCR	1.686	1.658	1.710	1.663	0.02	0.132
Experiment 2						
Initial weight (g)	41.54	41.49	41.53	41.55	0.05	0.994
Final weight (g)	2231.63^{b}	2547.45^{a}	2400.24^{a}	2530.72^{a}	44.07	0.003
Weight gain (g)	1909.37^{b}	2094.93^{a}	2159.11^{a}	2181.97^{a}	37.78	0.022
Feed intake (g)	3162.16^{b}	3635.88^{a}	3762.76^{a}	3808.36^{a}	91.06	0.002
FCR	1.656	1.703	1.736	1.745	0.02	0.137

SEM, standard error of mean; [a,b]values in a row with no common superscripts differ significantly(P < 0.05); C, control; A, Arginine; G, glutamine; T, tryptophan

Table 2: Effect of different amino acid on meat quality of broiler chicks

Meat quality properties		Treatments				SEM	P value
		C	A	G	T		
Experiment 1							
Live weight (g)		2123^{b}	2547^{a}	2397^{a}	2530^{a}	26.17	0.003
Warm carcass weight (g)		1559^{b}	1888^{a}	1748^{a}	1877^{a}	23.38	0.019
Breast muscle weight (%)		17.01	17.71	17.81	17.82	1.73	0.241
Thigh muscle weight (%)		10.95	11.62	11.97	12.39	1.29	0.416
pH		5.94^{b}	6.02^{b}	5.97^{b}	6.43^{a}	0.02	0.003
Meat color (CIE)	L* values	53.6^{a}	54.00^{a}	53.06^{a}	51.67^{b}	0.42	0.036
	a* values	2.18^{b}	3.23^{ab}	3.52^{ab}	4.27^{a}	0.05	0.002
	b* values	6.54^{a}	6.38^{ab}	5.70^{b}	4.31^{c}	0.21	0.012
Experiment 2							
Live weight (g)		1950^{b}	2160^{a}	2206^{a}	2217^{a}	24.29	0.012
Warm carcass weight (g)		1352^{b}	1492^{a}	1521^{a}	1517^{a}	22.26	0.027
Breast muscle weight (%)		18.25	18.24	18.18	18.45	1.61	0.452
Thigh muscle weight (%)		10.97	11.15	10.92	11.01	1.13	0.271
pH		5.69^{b}	6.07^{a}	5.81^{ab}	5.90^{ab}	0.01	0.038
Meat color (CIE)	L* values	52.80^{a}	51.62^{b}	52.57^{a}	51.04^{b}	0.30	0.041
	a* values	3.78^{ab}	4.51^{a}	4.36^{a}	3.05^{b}	0.12	0.028
	b* values	9.34^{a}	8.77^{b}	8.99^{ab}	6.99^{b}	0.16	0.021

SEM, standard error of mean; [a,b]values in a row with no common superscripts differ significantly (P < 0.05); C, control; A, Arginine; G, glutamine; T, tryptophan

Table 3: Effect of different amino acids on gut morphology of broiler chicks (Experiment 1)

Treatments	C	A	G	T	SEM	P value
Jejunum						
Villus height (µm)	59.25^{b}	69.79^{a}	64.39^{ab}	65.92^{a}	3.42	0.027
Crypt depth (µm)	18.26	16.90	17.33	17.43	1.81	0.432
Villus height-to-crypt depth ratio	3.51^{b}	3.99^{a}	3.78^{ab}	3.83^{a}	0.26	0.041
Ileum						
Villus height (µm)	44.38^{c}	52.28^{a}	49.53^{ab}	45.63^{bc}	2.98	0.018
Crypt depth (µm)	15.01^{a}	13.45^{bc}	14.69^{ab}	12.23^{c}	1.69	0.034
Villus height-to-crypt depth ratio	3.09^{b}	3.49^{ab}	3.85^{a}	3.75^{ab}	0.18	0.0329

SEM, standard error of mean; [a,b]values in a row with no common superscripts differ significantly (P < 0.05); C, control; A, Arginine; G, glutamine; T, tryptophan; Expt, experiment

Table 4: Effect of different amino acid on immune responses of broiler chicks (Experiment 2)

Treatment	C	A	G	T	SEM	P value
IgG (mg/ml)	158.96	213.79	196.16	192.23	14.27	0.635
IgA (µg/ml)	297.709	367.06	366.90	316.96	15.10	0.248

SEM, standard error of mean; C, control; A, Arginine; G, glutamine; T, tryptophan; IgG, Immunoglobulin G; IgA, Immunoglobulin A

Conclusion

Based on two experiments, dietary supplementation of L-Arg, L-Trp or L-Gln at 0.5% improves growth, gut morphology and meat quality of broiler chicks. Diets supplemented with 0.5% Arg, Gln or Trp provided a better development of the intestinal mucosa in broiler chickens. Thus the addition of Arg, Trp or Gln each at 0.5% in the diet of broiler chicken enhanced growth performance, immunity, gut morphology and meat quality characteristics of broiler.

Conflicts of Interest

The authors declare that there are no potential conflicts of interests.

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