Dietary Supplementation of Purified Amino Acid Derived from Animal Blood on Immune Response and Growth Performance of Broiler Chicken

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ABSTRACT

The existences of protein are important to supply nutritional requirements and to support optimal growth performance in modern broiler chicken. The present experiment was conducted to evaluate the effect of purified amino acid (PAA) isolated from animal blood on growth performance and immune response. A total of one hundred of 1-day old broiler chicken were used in the experiment, following a completely randomized design of 4 groups of treatment differed in concentrations of PAA supplementation (T1: control, no PAA addition; T2: 0.05%; T3: 0.1%; and T4: 0.5%) with 4 replicates for each group. Levels of various cytokines, such as IgA, IgG, interleukin (IL)-2, IL-6, tumor necrosis factor α, and interferon γ, were analyzed using an ELISA kit. Insulin-like growth factor 1, an important growth hormone, was also examined using an ELISA kit. The present result showed feed efficiency and average daily feed intake of broiler chicken increased significantly along with increasing concentrations of PAA ($P<0.05$). Plasma biochemical parameters and carcass traits were affected by supplementation of PAA. Insulin growth factor-1 levels were significantly increased along with increasing concentrations (until 0.5%) of PAA ($P<0.05$). Inflammatory marker levels significantly differed between the control and treatment groups. These results indicate that purified amino acid isolated from animal blood affected the immune response and growth performance of broiler chicken. Furthermore, PAA can be used in feed supplementation for broiler chickens without causing health issues. It suggests that the beneficial impacts of PAA on immune responses, as well as blood characteristics, may improve broiler performance.

Keywords: animal blood, broiler chicken, growth performance, immunomodulatory, purified amino acid

INTRODUCTION

Animal feed supplementation is used in the livestock industry for many different reasons, such as to reduce the cost of production, as feed is a major cost factor in the livestock industry (Rayani *et al.*, 2017; Reyes *et al.*, 2018). This is also true for the poultry industry in South Korea. According to the Korea Statistical Information Service, the cost of poultry production accounts for 61% of the total livestock production cost in 2015. Numerous studies have evaluated feed for reducing production costs. In Korea, utilization of natural extracts, such as wild-ginseng, green tea, fruit extract, and red pepper seed oil, had been evaluated as feed supplementation for broiler chicken in order to reduce cost production (An *et al.*, 2007; Kim *et al.*, 2014; Yang *et al.*, 2003; Yan *et al.*, 2011).

Beside of low cost production, the health status of animals with high growth performance is also important in the choice of feed supplementation. Feed supplementation with high-quality protein has been used chiefly to improve the growth performance and immune system in animals (Fisher, 1998; Seifdavati *et al.*, 2008; Sklan & Noy, 2004).

Local protein sources such as blood meal may be useful for reducing production costs. Moreover, livestock are considered as one cause for environmental pollution. Blood meal is a by-product of animal slaughter and can be used as a protein source in animal diets, such as in ruminants and broiler chickens (McDonald *et al.*, 1992; Khawaja *et al.*, 2007). Generally vegetable protein supplements are deficient in two important essential amino acids for poultry, i.e. lysine and methionine, whereas blood meal is rich in both of these amino acids (Konashi *et al.*, 1999; Sklan & Noy, 2004). Purified amino acids isolated from animal blood were likely used to increase the quality of protein in basal diet (Ambardekar *et al.*, 2009); moreover these amino acids can reduce production costs by improving the quality of primary protein source in poultry diets. The importance of protein in feed formulation is more evident because it is the most costly nutrient in feeds is and dietary crude protein (CP) requirement is high in modern broiler strains as a result of their fast growth rates (Abbasi *et al.*, 2014).

Many studies have been conducted to show the important of individual amino acid effects to support optimal growth performance in modern broiler (Han *et al.*, 1992; Aletor *et al.*, 2000; Kim *et al.*, 2007).

During the last few years, mostly blood meal is being used as feed supplementation in broiler chicken (Seifdavati *et al.*, 2008). A long time ago, highly purified amino acid supplementation also significantly increased the growth performance and immune reactivity of rats (Bounous & Kongshavn, 1977). Little information is available of purified amino acid use in broiler diets. Because of lack information about purified amino acid supplementation in broiler chicken, especially in South Korea, this present study was conducted to evaluate its effect on the immune response, growth performance, carcass performance, and blood characteristics of broiler chicken.

MATERIALS AND METHODS

Preparation of Purified Amino Acid

Waste blood generated from an animal slaughterhouse was collected under hygienic conditions. Proteins content in collected blood were separated into amino acids by enzymatic decomposition. The product of enzymatic decomposition was dehydrated and sterilized. Thus, the amino acid produced had low moisture content and free from microorganisms. Nutrients content of purified amino acid used in the present study is shown in Table 1.

Table 1. Nutrients content of purified amino acid used in the experiment

Nutrients	Contents
Moisture, (%)	3.790
Crude protein, (%)	93.370
Ether extract, (%)	0.840
Crude fiber, (%)	0.390
Gross energy (kcal kg^{-1})	5374.00
Calcium (mg kg^{-1})	406.63
Phosphorous (mg kg^{-1})	1676.12
Cysteine, (%)	1.041
Methionine, (%)	0.458
Aspartate, (%)	10.576
Threonine, (%)	3.407
Serine, (%)	4.565
Glutamine, (%)	8.875
Glycine, (%)	3.787
Alanine, (%)	6.602
Valine, (%)	6.483
Isoleucine, (%)	0.835
Leucine, (%)	11.654
Tyrosine, (%)	2.100
Phenylalanine, (%)	6.219
Lysine, (%)	7.313
Histamine, (%)	5.703
Arginine, (%)	3.809
Proline, (%)	2.674

Animal and Experimental Design

This experiment was conducted in an animal research farm facility in Anseong, South Korea. All the animal care management and procedures were approved by the Hankyong National University Animal Care and Use Committee. A total of 100 day old male broiler chicken (Ross) were housed in electrically heated battery brooders and fed a commercial starter diet for adaptation until day 7. The birds were randomly distributed in four treatment groups with four pens per treatment (6-7 birds per pen). Nutrient composition of basal diet used in this experiment presented in Table 2. The mortality rate in this study was 10% for the 35 days growing period. They were fed to diets treatments twice per day and provided with fresh water *ad-libitum*. Diet treatments were prepared by purified amino acid as top dressing of the basal diet as shown in Table 3.

Analyses of Blood Parameters and Serum Immunoglobulin

Blood samples were collected, on days 20 and 35. Plasma samples were separated by centrifugation for 15 min at 5000 x*g* at 4° C, and then stored at -70° C until further analysis. Plasma biochemical parameters, such

Table 2. Nutrient composition (%) of broiler basal diets used in the experiment

Item	Basal starter	Basal grower	Basal finisher
Ingredients			
Corn	52.2	55.9	61.7
Wheat bran	2.7	2.5	2.1
Soybean meal	34.1	31.6	27.2
Corn gluten meal	4.5	3.8	2.9
Soybean oil	2.5	2.5	2.5
Limestone	1.1	1.0	1.0
Dicalcium phosphate	1.3	1.25	1.1
Salt	0.25	0.25	0.25
L-Lysine	0.1	0.1	0.1
DL-Methionine	0.25	0.2	0.15
Premix	1.0	1.0	1.0
Nutrient composition			
ME (kcal/kg)	3052	3104	3154
CP (%)	23.1	21.3	19.4
Lysine (%)	1.22	1.1	1.0

Table 3. Experimental design for diet treatments

Treatments	Diets
Treatment 1 (T1)/ Control	Basal diet[1]
Treatment 2 (T2)	Basal diet + 0.05% Purified amino acid
Treatment 3 (T3)	Basal diet + 0.1% Purified amino acid
Treatment 4 (T4)	Basal diet + 0.5% Purified amino acid

[1]Basal diet: Day 1-10=broiler starter (crude protein: 23.1% and metabolizable energy: 3052 kcal kg^{-1}); Day 11-20=broiler grower (crude protein: 21.3% and metabolizable energy: 3104 kcal kg^{-1}); Day 21-35=broiler finisher (crude protein: 19.4% and metabolizable energy: 3154 kcal kg^{-1}).

as glucose, total cholesterol, total bilirubin, glutamate-oxaloacetate transaminase, and blood urea nitrogen, were measured according to AOAC (1990) to evaluate the overall health condition of the broiler chicken. Plasma biochemical levels were determined using an automated biochemical analyzer, Spotchem EZ SP-4430. Plasma levels of inflammatory markers (IgA (CSB-E11232Ch), interleukin (IL)-2 (CSB-E06755Ch), IL-6 (CSB-E08549Ch), tumor necrosis factor (TNF)α (CSB-E11231Ch), and interferon (IFN)γ (CSB-E08550Ch)) and the muscle development marker insulin-like growth factor-1 (IGF-1) (CSB-E09867Ch) were evaluated by ELISA kit (Cusabio Biotech Co., Ltd., Wuhan, China). In addition, IgG (LS-F4752) were purchased from LifeSpan BioSciences, Inc., Seattle, WA, USA. The experiments were conducted according to the manufacturer's instruction.

Growth Performance

To determine the daily feed intake and feed efficiency, the feed supply and leftover feed in each pen were recorded every day. Birds were weighted once a week to determine the body weight gain of the birds.

Carcass Traits and Relative Organ Weights

At the end of the experimental period, four birds from each treatment were randomly selected, fasted overnight, and sacrificed for carcass analysis. All carcass traits and relative organ weights were expressed as a percentage of the live weights (Seifdavati et al., 2008).

Statistical Analyses

Data are shown as the mean ± SD (standard deviation). Differences between groups were analyzed by one-way analysis of variance, followed by Duncan's multiple range test. The level of statistical significances was set at $P < 0.05$. The statistical software package SPSS 15.0 (SPSS, Inc., Chicago, IL, USA) was used for the analysis.

RESULTS

Inflammatory markers were detected in blood plasma during the grower and finisher periods (Table 4). Some parameters were affected by purified amino acid supplementation, such as IgA, IgG, IL-2, IL-6, IFNγ, and TNFα. The parameters were significantly different (either higher or lower) between the control and treatment groups ($P < 0.05$). The result of immunoglobulin levels showed that purified amino acid supplementation in the treatment group decreased inflammation in the broiler chickens. IL-2 and IL-6 levels decreased along with increasing levels of purified amino acid, indicating that supplementation with purified amino acids had a positive immunomodulatory effect in the birds. Levels of IFNγ and TNFα were significantly different between the control and treatment groups. Levels of IGF-1 were increased along with increasing concentrations of purified amino acid supplementation.

To evaluate the overall health condition of broiler chickens, plasma biochemical analysis was conducted. As shown in Table 5, all parameters were within the normal ranges, indicating that purified amino acid supplementation had no negative effects on the broiler chickens. Glucose and total cholesterol levels of broiler chickens at grower period were significantly different from those in controls ($P < 0.05$). Blood urea nitrogen was also measured to observe protein digestibility in the broiler chicken. Blood urea nitrogen levels in the plasma were less than 5 mg/dL which indirectly indicated that the digestibility of purified amino acid was high.

The averages of body weight, feed consumption, and efficiency are presented in Table 6. Birds supplemented with purified amino acid showed higher body weights than control birds, but the different was not significant between the control and treatment groups. Average daily gain and average daily feed intake were also not statistically significant ($P < 0.05$). The average daily gain of birds in the T4 group was higher than in the control group, whereas the average daily feed intake was significantly lower than the control birds ($P < 0.05$). Feed efficiency in this study increased along with increasing purified amino acid contents as shown in Table 6 ($P < 0.05$). The feed efficiency of birds in the T4 group was the highest value among all treatments. Improving average daily gain is a standard method for measuring growth performance. However, improving feed efficiency is also important for increasing profitability. Table 7 shows that the weight of the carcass, breast, and thigh were affected by increasing the concentrations of purified amino acid ($P < 0.05$), while the liver and viscera

Table 4. Immune levels of broiler chicken fed diets supplemented with purified amino acid and reared up to 35 days of age

Variables	Grower (20d)				Finisher (35d)			
	T1	T2	T3	T4	T1	T2	T3	T4
IgA, (mg/dL)	80.04 ± 6.5[b1)]	93.77 ± 6.4[b]	75.38 ± 4.8[b]	31.02 ± 6.8[a]	22.03 ± 2.1[ab]	17.9 ± 4.9[a]	31.72 ± 1.3[b]	20.93 ± 1.3[ab]
IgG, (mg/dL)	2.89 ± 0.5[b]	2.16 ± 0.1[a]	2.18 ± 0.2[a]	2.66 ± 0.7[ab]	2.067 ± 0.2	2.067 ± 0.2	2.067 ± 0.2	2.067 ± 0.2
IL-2, (mg/dL)	0.329 ± 0.4[b]	0.181 ± 0.1[a]	0.301 ± 0.3[b]	0.192 ± 0.1[a]	0.905 ± 0.7[b]	0.173 ± 0.1[a]	0.213 ± 0.1[a]	0.556 ± 0.6[ab]
IL-6, (mg/dL)	12.894 ± 2.8[b]	11.48 ± 1.7[ab]	8.996 ± 1.5[a]	8.586 ± 1.0[a]	4.91 ± 0.2	4.90 ± 1.6	4.73 ± 0.9	4.26 ± 0.2
TNF α, (mg/dL)	2.727 ± 3.8	1.771 ± 2.0	3.552 ± 2.1	2.97 ± 1.4	35.10 ± 2.7[b]	9.71 ± 9.4[a]	0.81 ± 0.7[a]	1.60 ± 1.7[a]
IFN γ, (mg/dL)	25.13 ± 1.3[b]	5.938 ± 4.2[a]	1.5795 ± 2.3[a]	1.554 ± 1.0[a]	554.86 ± 0.1[b]	410.06 ± 0.2[ab]	348.82 ± 0.1[ab]	253.92 ± 0.2[a]
IGF-1, (mg/dL)	852.65 ± 9.4[a]	861.77 ± 6.3[a]	865.71 ± 6.8[a]	938.06 ± 2[b]	705.03 ± 2.8[a]	774.13 ± 0.2[a]	1003.21 ± 9.7[b]	1058.67 ± 4.8[b]

[1)] Means ± SD values with different superscripts are significantly different ($P < 0.05$).
T1: control, no purified amino acid (PAA) addition; T2: control + 0.05% PAA; T3: control + 0.1% PAA; and T4: control + 0.5% PAA.

were not significantly affected. Birds in the T4 group showed the highest weight in the carcass and breast, while birds in the T1 group showed the highest thigh weight.

DISCUSSION

Blood meal is used as a source of crude protein to increase the crude protein content of basic diets. Generally, the crude protein content in blood meal was 81.1% (NRC, 1994). The purified amino acid used in this study was derived from blood meal and the purification process increased the crude protein content tremendously. Dietary protein was very important for the normal growth and reproduction of animals (Han & Lee, 2000; Abdurrahman *et al.*, 2016). Many studies (Hansen & Lewis, 1993; Tuitoek *et al.*, 1997) have demonstrated a protein sparing effect of using amino acid supplementation to balance low-crude protein diets in monogastric animals. Purified amino acid contained substantial amounts of essential amino acids, such as lysine (7.313%), leucine (11.654%), and aspartate

(10.576%). Aspartic acid has immunomodulatory functions, as it enhances immunoglobulin production and antibody formation (cOoi & Liu, 2000; Liu *et al.*, 2007). The higher content of purified the amino acids in the diet may result in a better amino acid digestibility and lead to a better performance compare to crude protein (Abbasi *et al.*, 2014).

Amino acids contained in the diets are fundamental because it is related to tissue synthesis. Andrews *et al.* (1995) stated that tissue synthesis is the synthesis of complement as an immune regulator. There is much evidence that essential amino acids modulate immune functions in broiler chicken (Li *et al.*, 2007). Protein has recommended values in the antibody signaling (Abbasi *et al.*, 2014). Immunoglobulin levels were also measured to evaluate immune system function. Dietary protein especially amino acid influences immune system components by decreasing serum IgA and IgG levels (Abbasi *et al.*, 2014). Those two immunoglobulins were also related to intestine health of broiler chicken. IFNγ and TNFα play an important role in inflammation. Low secretion of those cytokines signifies low inflammatory stimuli in

Table 5. Plasma biochemical analysis of broiler chicken fed diets supplemented with purified amino acid and reared up to 35 days of age

Variables[1]	Grower (20d)				Finisher (35d)			
	T1	T2	T3	T4	T1	T2	T3	T4
Glucose, (mg/dL)	238.67 ± 1.56	226.33 ± 2.89	236.67 ± 0.21	254 ± 1.36	200 ± 4.353[a]	225 ± 2.376[ab]	234 ± 2.957[b]	286.25 ± 0.178[c]
Total cholesterol, (mg/dL)	150 ± 4.11	152.33 ± 1.33	140 ± 1.3	131 ± 1.73	133.25 ± 5.679[a]	175.5 ± 2.294[b]	140.25 ± 4.878[a]	128.5 ± 2.863[a]
Total bilirubin, (mg/dL)	0.13 ± 0.06	0.2 ± 0.2	0.167 ± 0.1	0.57 ± 0.3	0.225 ± 0.189	0.275 ± 0.171	0.225 ± 0.263	0.15 ± 0.238
GOT, (mg/dL)	119.3 ± 27.4	115 ± 11.3	105.7 ± 24.6	127.3 ± 5.5	214.75 ± 3.3	196.25 ± 1.556	219.25 ± 4.909	307.75 ± 8.991

[1] Normal values for the plasma biochemistry parameters used in the study (The Merck Veterinary Manual, 10th Ed, 2010): Glucose: 180-400 mg/dL; Total cholesterol: 129-297 mg/dL; Total bilirubin: 0-0.1 mg/dL; GOT: 10-400 IU/L.
Means with different superscripts are significantly different (P<0.05).
T1: control, no purified amino acid (PAA) addition; T2: control + 0.05% PAA; T3: control + 0.1% PAA; and T4: control + 0.5% PAA.

Table 6. Growth performance of broiler chicken fed diets supplemented with purified amino acid and reared up to 35 days of age

Variables	T1	T2	T3	T4
Average initial body weight, (g/chick)	133.90 ± 0.2	133.50 ± 0.3	133.30 ± 0.2	135.00 ± 0.2
Average final body weight, (g/chick)	2962.50 ± 0.6	3062.50 ± 0.7	3033.30 ± 0.7	3020.80 ± 0.6
Average daily gain, (g/chick/day)	80.77 ± 0.2	83.72 ± 0.2	82.86 ± 0.2	82.45 ± 0.2
Average daily feed intake, (g/chick/day)	134.98 ± 0.3[b]	130.12 ± 0.4[ab]	131.35 ± 0.4[ab]	121.33 ± 0.3[a]
Feed efficiency (g of weight gain/g of feed intake)	0.599 ± 0.04[a]	0.643 ± 0.08[b]	0.631 ± 0.04[b]	0.68 ± 0.07[c]

Means values with different superscripts are significantly different (P<0.05).
T1: control, no purified amino acid (PAA) addition; T2: control + 0.05% PAA; T3: control + 0.1% PAA; and T4: control + 0.5% PAA.

Table 7. Performance of carcass of broiler chicken fed diets supplemented with purified amino acid and reared up to 35 days of age

Variables	T1	T2	T3	T4
Carcass, (% of body live weight)	72.89 ± 7.78[b]	65.63 ± 3.19[a]	76.72 ± 11.66[c]	76.82 ± 6.01[c]
Breast, (% of body live weight)	25.94 ± 0.76[b]	24.49 ± 2.56[a]	29.06 ± 4.69[c]	29.39 ± 2.95[c]
Thigh, (% of body live weight)	16.38 ± 0.91[b]	14.38 ± 0.64[a]	16.10 ± 1.45[b]	15.31 ± 1.50[ab]
Liver, (% of body live weight)	2.49 ± 0.46	2.25 ± 0.57	2.11 ± 0.18	2.43 ± 0.68
Viscera, (% of body live weight)	8.10 ± 0.76	8.42 ± 1.02	8.27 ± 1.8	8.47 ± 0.96

Means with different superscripts are significantly different (P<0.05).
T1: control, no purified amino acid (PAA) addition; T2: control + 0.05% PAA; T3: control + 0.1% PAA; and T4: control + 0.5% PAA.

the body (Duque & Descoteaux, 2014). IGF-1 concentrations, a key regulator of muscle development, have been demonstrated to depend on the level of dietary protein intake (Wheelhouse *et al.*, 1999). Furthermore, amino acid deposition in skeletal muscle represents 65% of whole daily protein intake (Macari *et al.*, 1994). It showed that amino acid is necessary to be considered for bird requirements compared to crude protein. Our findings support that purified amino acid supplementation enhances the primary immune responses of broiler chicken. Further studies are needed to clarify the specific effects of purified amino acid supplementation on intestine morphology in broiler chicken.

The present study demonstrates that the growth of broiler chicken is affected by increasing dietary supplementation of purified amino acid from 0.05 to 0.5%. Broiler chickens decreased feed intake as protein content in the diet increased (Lesson *et al.*, 1996). Those previous study are consistent with our findings which also decreasing in feed intake along with the increasing levels of purified amino acid. Furthermore, dietary supplementation of high-protein amino acid decreased feed consumption with a concomitant decrease in carcass fat deposition (Rosebrough & McMurtry, 1993). Aletor *et al.* (2000) mentioned that decreasing dietary protein in broiler chicken generally tended to decrease the relative weight of liver and viscera fat. Our results showed that the increasing levels of purified amino acid supplementation also increased carcass fat deposition. In addition, relative weight of liver and viscera were not significantly affected by purified amino acid.

CONCLUSION

Purified amino acid supplementation had positive immunomodulatory effects in broiler chicken, and thus is a valuable protein supplement for increasing chicken production without causing side effects. Increasing concentrations of purified amino acid supplementation increased the growth performance and muscle development of broiler chicken. Improving the health status leads to enhance growth performance of broiler chicken. Furthermore, the utilization of animal by-products can reduce production costs and environmental pollution.

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