Effects of lysine, methionine, threonine and tryptophan on growth performance and serum antibody titers to Gumboro disease of color-feathered chickens

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ABSTRACT

The experiment was conducted to determine the effects of four amino acids (lysine, methionine, threonine and tryptophan) supplementation on growth performances and serum antibody titers to Gumboro disease (IBD, infectious bursal disease) in broiler chickens. Chicks were randomly assigned to 5 dietary groups (5 chicks/group as 12 replicates of 5 chicks) in a complete randomised experimental design. Group I served as control group, was fed a diet without any supplementation. Groups II and III were supplemented 10% of four amino acids (lysine, methionine, threonine and tryptophan) of the recommended requirements for 45 and 42 days, respectively. Meanwhile, Groups IV and V were supplemented 20% of four amino acids (lysine, methionine, threonine and tryptophan) of the recommended requirements for 45 and 42 days, respectively. The supplementation was started at 3 days of age in chicks. The chickens were vaccinated against Gumboro disease at day 12 and day 19. The antibody titer of the chickens in each group was assayed using IBD anti-body ELISA. Besides, the live body weight, average feed intake and feed conversion (FCR) were determined at 0, 21, 42 and 84 days of age. The results showed no significant different between groups in performance parameters such as body weight and FCR. However, the present work showed highest dose of lysine, methionine, threonine and tryptophan supplementation for 42 days may be improved feed intake and immune response of chicken against Gumboro disease vaccination.

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1. Introduction

In order to be effective in chicken production, preventive medicine, genetic selection and improved nutrition and management should be concerned carefully. Nutritional supplements (carbohydrate, protein and fats) should be provided to ensure the growth, repair of damaged tissues as well as daily maintenance. However, deficiency or excess of dietary protein or amino acids alters immune responses (Payne et al., 1990). Infectious bursal disease (or Gumboro disease) is one of the economically most important diseases that affects commercially produced chickens worldwide (Eterradossi & Saif, 2008). Chickens infected with IBDV between 3 and 6 weeks of age mostly show clinical signs and mortality accompanied with bursal atrophy. According to Muller et al. (2003), strain and the amount of the virus, age and the breed of chickens, the route of inoculation, the presence or absence of neutralizing antibodies, intercurrent primary and secondary pathogens and environmental and management factors affect the level of serious clinical signs in chicken. In chicken, infected with IBDV can cause immunosuppression, which makes the birds vulnerable to a variety of secondary infections chickens also develop a poor immune response to vaccination against other pathogens (Mazariegos et al., 1990). Therefore, strict hygiene management and vaccination programmes have been used to prevent IBD. For optimal growth and immune response, lysine and methionine are required for protein synthesis by mammals and avian species (Rubin et al., 2007). Tryptophan can be considered as a third limiting amino acid for poultry, followed by methionine and lysine (Peganova et al., 2003). According to Kidd & Hackenhaar (2006), tryptophan deficiency not only affects carcass quality but it also impairs the synthesis of important neurotransmitters such as serotonin and melatonin. Besides, threonine is a major component of intestinal mucin and plasma gamma-globulin in animals (Kim et al., 1999). Thus, the aims of this study was to evaluate the effects lysine, methionine, threonine and tryptophan requirements on growth performances and serum antibody titer to Gumboro disease in broiler chickens.

2. Materials and Methods

A total of 300 day-old chicks (DOC) of color feather breed named "Huynh De" were obtained from Binh Minh breeder farm to use in all experiments. The chicks were weighed on arrival, and randomly allocated into 5 equal groups with 12 replicates each (5 chicks per replicate). Feed and water were provided ad libitum. The basal diet (Table 1) was formulated to contain all essential amino acids at recommended levels (NRC, 1984), modified by Duong Duy Dong (unpublished materials). In addition, chemical composition of the basal diet used in chicken feeding was shown in Table 2. Group I served as control group, was fed a basal diet without any supplementation. Groups II and III were supplemented 10% of four amino acids (lysine, methionine, threonine and tryptophan) of the recommended requirements for 45 and 42 days, respectively. Meanwhile, Groups IV and V were supplemented 20% of four amino acids of the recommended requirements for 45 and 42 days, respectively. The supplementation was started at 3 days of age in chicks. The chicks were vaccinated against Newcastle disease on 4, 20 and 42 days of age; and Gumboro diseases on 12 and 19 days of age. Response variables measured during the experiment included body weight, body weight gain; feed intake, feed conversion ratio on day 0, 21 42 and 84 days of age. On 11, 18, 27, 34, 41, 49, 56, 63, 70, 77 and 84 days of age, five birds from each group were chosen at random and blood samples were collected from the brachial vein. Serum was separated by centrifugation (3000 g, 15 min) and antibody titre against IBD were performed using commercially available ELISA kits (IDEXX, Labs Inc., Westbrook, Maine, USA) according to manufacturer's instructions. The data obtained were analyzed by Tukey's test and one-way analysis of variance (ANOVA) using Minitab 16.0.A P value < 0.05 was considered statiscally significant.

3. Results and Discussion

3.1. Growth performance

As shown in Table 3, the body weight and average daily gain of Group III was higher than those at 84 days of age. However, no significant difference about the body weight and average weight gain was found among treatment Groups. The body weights of this study reached the standards of Binh Minh company in which chickens at 100 days of age were gained from 1.7 to 1.9 kg (Vu, 2015). The highest food consumption in Group I (control group) and the lowest of this found in Group IV with the increment level of 20% for 45 days of 4-amino acid mixture supplementation were also found. Compared with Control Group, Groups I and II, it was observed that dietary treatment Groups IV and V had significant effects on the feed intake ($P < 0.001$) and feed conversion ratio ($P < 0.05$). Bouyeh (2012) also confirmed that the increment levels of 10%, 20% and 30% lysine and methionine would increase body weight, cardiac and liver weight as well as decrease feed intake of chicken. On the contrary, the increment level of 40% of these two amino acids would decrease body weight of chicken. Rogers & Pesti (1990) also reported that tryptophan-deficient diets caused a reduction in weight gain; but, the excess tryptophan in the diet caused a numerical decrease in bird weight gain (Koelkebeck et al., 1991). Therefore, the standard and the amount of amino acid consumed by the birds have a great influence on weight gain and feed intake (Teeter et al., 1993).

Table 1. Ingredient composition of the experiment diets (basal diet)

Ingredients (%)	0 - 21 days of age	22 – 42 days of age	43 – 84 days of age
Corn	63.219	63.513	62.930
Rice bran I	5.791	2.387	28.796
Soybean meal 46	23.175	26.129	2.500
Fish oil	2.460	2.500	0.353
L-Lysin-HCl 98%	0.212	0.135	0.790
DL-Methionin	0.210	0.249	0.249
L-Threonin 98.5	0.100	0.210	0.117
L-Tryptophan 98	0.029	0,097	0.035
$NaHCO_3$	0.817	0.820	0.805
Choline chloride 60	0.120	0.120	0.120
Antioxidants	0.020	0.020	0.020
Limestone powder	1.186	1.170	1.157
Premix BA112	0.250	0.250	0.250
Poison absorption	0.100	0.100	0.100
Herb extracts	0.015	0.015	0.015
Precursor creatine	0.060	0.060	0.060
DCP 18	2.086	2.077	2.065
Organic acids	0.100	0.100	0.100
Probiotics	0.050	0.050	0.050

Table 2. Chemical composition of the basal diet used in chicken feeding

Composition	Unit	0-21 days of age	22 – 42 days of age	43 – 84 days of age
Dry matter	%	87.699	87.628	87.581
Metabolisable energy	Kcal/kg	2900.000	2900.000	2900.000
Crude protein	%	19.000	18.000	17.000
Crude lipid	%	5.614	5.651	5.811
Linoleic acid	%	1.367	1.259	1.179
Gross fiber	%	2.911	2.975	3.086
Total mineral	%	5.805	5.899	6.052
Calcium	%	0.950	0.950	0.950
Total phosphorus	%	0.703	0.719	0.743
Available phosphorus	%	0.400	0.400	0.400
Sodium	%	0.226	0.230	0.230
Chlorides	%	0.140	0.134	0.128
dEB	meq	240.000	240.000	240.000
Total lysine	%	1.174	1.090	0.996
Total methionine	%	0.538	0.489	0.477
Total Met. + Cys.	%	0.857	0.797	0.773
Total threonine	%	0.813	0.758	0.724
Total tryptophan	%	0.235	0.189	0.205
Digestible lysine	%	1.080	1.000	0.910
Digestible methionine	%	0.512	0.464	0.452
Digestible Met.+ Cys.	%	0.778	0.720	0.697
Digestible threonine	%	0.686	0.635	0.604
Digestible tryptophan	%	0.210	0.166	0.182
Digestible leucine	%	1.480	1.416	1.340
Digestible isoleucine	%	0.707	0.665	0.619
Digestible valine	%	0.777	0.738	0.695

Table 3. Body weight, average daily gain, feed intake and feed conversion ratio

Group	0 day of age	21 days of age	42 days of age	84 days of age
		Body weight (g)		
I	35.25 ± 0.96	221.33 ± 15.10	602.50^{ab} ± 35.20	1489.17 ± 96.90
II	35.75 ± 1.21	233.67 ± 12.35	617.50^{ab} ± 35.50	1502.50 ± 99.30
III	35.00 ± 1.04	218.17 ± 19.99	621.67^{a} ± 49.70	1520.83 ± 122.10
IV	35.66 ± 1.23	224.00 ± 15.63	575.00^{b} ± 34.25	1510.00 ± 112.20
V	35.83 ± 0.83	228.00 ± 16.88	570.83^{b} ± 56.00	1516.67 ± 91.10
P	0.263	0.172	0.011	0.952
Group	0 - 21 days of age	22 – 42 days of age	43 – 84 days of age	0 – 84 days of age
		Average daily gain (g)		
I	8.45 ± 0.68	18.15^{ab} ± 1.46	21.11 ± 2.27	17.10 ± 1.14
II	8.99 ± 0.53	18.27^{ab} ± 1.60	21.07 ± 2.01	17.25 ± 1.16
III	8.33 ± 0.88	19.21^{a} ± 2.35	21.40 ± 3.02	17.47 ± 1.43
IV	8.56 ± 0.68	16.71^{b} ± 1.19	22.26 ± 2.57	17.34 ± 1.31
V	8.73 ± 0.76	16.32^{b} ± 2.07	22.52 ± 2.13	17.42 ± 1.07
P	0.195	0.001	0.459	0.953
		Feed intake (g/day)		
I	19.03^{b} ± 2.42	41.72^{a} ± 2.64	69.20 ± 0.37	49.43^{a} ± 0.98
II	21.67^{a} ± 1.72	39.92^{b} ± 3.06	67.84 ± 2.55	48.99^{b} ± 1.88
III	18.01^{b} ± 1.52	39.07^{ab} ± 3.37	68.87 ± 1.71	48.34^{cb} ± 1.64
IV	18.97^{b} ± 2.43	34.64^{bc} ± 3.63	66.62 ± 1.54	46.38^{cb} ± 1.34
V	19.08^{b} ± 1.93	35.60^{c} ± 4.38	68.07 ± 5.23	46.68^{c} ± 1.49
P	0.001	0.000	0.199	0.000
		FCR (kg feed/kg weight gain)		
I	2.25 ± 0.27	2.30 ± 0.17	3.31 ± 0.36	2.90 ± 0.21
II	2.41 ± 0.21	2.19 ± 0.19	3.24 ± 0.29	2.85 ± 0.18
III	2.18 ± 0.28	2.07 ± 0.36	3.28 ± 0.52	2.78 ± 0.26
IV	2.23 ± 0.34	2.08 ± 0.29	3.02 ± 0.33	2.69 ± 0.19
V	2.19 ± 0.18	2.20 ± 0.29	3.04 ± 0.29	2.67 ± 0.17
P	0.216	0.207	0.217	0.049

[a-c]Mean values for control and amino-acid-supplement groups within a column not sharing a common superscript letter were significantly different at $P < 0.05$.

3.2. Serum antiboy titres against Gumboro disease in broiler chickens

According to IDEXX laboratories (2010), the antibody titres against Gumboro disease of around 1000-4000 would be sufficient to protect chickens from this disease. As shown in Table 4, the antibody titre against Gumboro disease after the first vaccination was lower than the minimum protective of 1000 in treatment Groups, except for the high antibody titre against Gumboro disease was also found in 18-day-old chicken of Group V (1099 ± 1144). The lower antibody titres following the primary vaccination could be due to the young age of chicken when the immune function of young animal is not well developed (Rubin et al., 2007). The higher antibody titre in Group IV was significantly different than those of the remaining Groups at 63 days of age. In addition, the antibody titre in Group IV was still higher than those of the other Groups at the end of this experiment; although, no significant difference was found. This observation was consent with the study conducted by Lidiya et al. (2015), the higher dose (140% of the recommended dose) of lysine and methionine improved immune response of chicken against infectious bursal disease vaccination. Furthermore, the increasing total methionine levels from 0.35 to 1.2% in the diet for chickens will enhance the aspects of the immune responses including T-cell proliferation in response to mitogen stimulation, plasma levels of immunoglobulin G; leucocyte migration and antibody titre (Swain & Johri, 2000). On the con-

Table 4. Serum antiboy titres against Gumboro disease in broiler chickens

Days of age	Item	Group I	Group II	Group III	Group IV	Group V	*P*
11	(X ± SD)	1277 ± 869	939 ± 310	563 ± 470	713 ± 365	854 ± 523	0.122
	CV (%)	68.02	33.05	83.38	51.19	61.28	
	Min	332	632	91	215	144	
	Max	2688	1543	1505	1334	1750	
18	(X ± SD)	400^{ab} ± 398	231.8^{b} ± 198.6	212.9^{b} ± 229.1	228.6^{b} ± 267.1	1099^{a} ± 1114	0.013
	CV (%)	99.56	85.71	107.61	116.84	101.35	
	Min	0	0.0	0.0	0.0	260	
	Max	1095	486.0	559.0	828.0	3200	
27	(X ± SD)	1900 ± 969	1764 ± 747	1726 ± 1171	2222 ± 1486	2181 ± 1985	0.915
	CV (%)	51.00	42.35	67.83	66.87	91.00	
	Min	574	626	130	639	0	
	Max	3134	2940	3339	4869	4946	
34	(X ± SD)	4019 ± 1354	3165 ± 1121	2889 ± 357	3177 ± 1621	4190 ± 1289	0.155
	CV (%)	33.69	35.40	12.36	51.02	30.77	
	Min	2466	1416	2489	1341	2003	
	Max	6323	5106	3569	5290	5919	
41	(X ± SD)	5163^{a} ± 2770	3559^{ab} ± 1463	2250^{b} ± 1529	3718^{ab} ± 919	2362^{b} ± 1271	0.110
	CV (%)	53.65	41.12	67.93	24.71	53.79	
	Min	1730	2030	144	2161	358	
	Max	9752	5459	4901	4994	4248	
49	(X ± SD)	4470 ± 2283	4874 ± 1733	3184 ± 1440	3694 ± 830	4611 ± 1201	0.198
	CV (%)	51.06	35.56	45.23	22.46	26.05	
	Min	2191	1760	307	2161	2891	
	Max	9003	6952	4625	4917	6565	
56	(X ± SD)	5146 ± 1187	3951 ± 1970	4240 ± 1721	3992 ± 1182	4567 ± 1698	0.576
	CV (%)	23.06	49.85	40.59	29.62	37.19	
	Min	2883	1083	1604	2298	2815	
	Max	6119	6494	6439	5941	7780	
63	(X ± SD)	4328^{ab} ± 1679	3008^{ab} ± 1565	2819^{b} ± 2021	5542^{a} ± 2281	3501^{ab} ± 1526	0.033
	CV (%)	38.80	52.02	71.71	41.17	43.58	
	Min	1899	435	59	3131	1279	
	Max	7347	5534	5413	8607	6073	
70	(X ± SD)	3494 ± 1770	3598 ± 1152	3134 ± 1639	5298 ± 2741	3547 ± 1599	0.178
	CV (%)	50.65	32.02	52.31	51.74	45.09	
	Min	1706	2124	1418	3278	231	
	Max	6775	5229	6736	11526	5472	
77	(X ± SD)	3030 ± 1742	3111 ± 1445	2753 ± 2473	4982 ± 2719	3719 ± 1008	0.192
	CV (%)	57.50	46.43	89.86	54.59	27.11	
	Min	0	971	324	1655	1529	
	Max	5336	4715	7515	10121	4702	
84	(X ± SD)	2983 ± 1482	2950 ± 1487	3256 ± 2399	3864 ± 2469	3191 ± 1416	0.878
	CV (%)	49.69	50.42	73.69	63.89	44.38	
	Min	1464	693	379	1548	242	
	Max	6404	5652	7270	8802	4869	

$^{a\text{-}c}$Mean values for control and amino-acid-supplement groups within a row not sharing a common superscript letter were significantly different at $P < 0.05$.

trary, the high supplemental levels of methionine or cysteine (1.45% in the diet) were detrimental to the growth and immune responses of chickens (Tsiagbe et al., 1987), probably due to the excess production of highly toxic substances (e.g. homocysteine and sulphuric acid) (Wu & Meininger, 2002). Besides, the antibody responses and cell-mediated immunity in chickens were reduced by the lack of dietary lysine (Chen et al., 2003).

In conclusion, the present study showed that the supplementation of 20% of lysine, methionine, threonine and tryptophan for 42 days seemed better in improving feed consumption, feed conversion ratio and antibody titre against Gumboro disease. Furthermore, evaluation of the effect of these amino acids under various management systems and in different breeds is recommended.

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