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ANIMAL GENETICS AND BREEDING

ANIMAL FEEDS AND NUTRITION

ANIMAL PRODUCTION

SCIENTIFIC NEWS

INFLUENCES OF LYSINE LEVELS ON MEAT YIELD, QUALITY AND ABILITY AGAINST INFLUENZA IN AC BROILERS

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ABSTRACT

The aim of this study was to evaluate the effects of different Lysine levels on growth, carcass composition and immunological response to bird flu vaccination in Ac chicken. Four hundred fifty one day-old chicks and two hundred forty of twenty eight day-old chicks were arranged in a completely randomized design with 5 diets (different lysine contents of 0, 5, 10, 15 and 20% of the diet) and 3 replicates. Birds were fed *ad libitum* and had free access to water. Blood samples were collected to analyze immunological responses to H_5N_1 vaccination at 28 and 56 day-old. Six birds per a treatment were slaughtered to evaluate bodyweight, carcass composition and chemical composition. Results showed that feed consumption of birds was similar (99.2-111.9 g/bird/week) when increasing dietary lysine, but bodyweight gain and feed conversion ratio were improved ($P<0.05$) in diet with 10% lysine inclusion (52.4 g/bird/week and 2.09kg feed/kg gain, respectively). In addition, bodyweight, carcass composition and amino acid content of breast muscle of Ac chicken were not different ($P>0.05$) between treatments, but ratio of weight of organs producing immunological antibodies of chicken was greater in the high Lysine diets.

Keywords: *Antibody, growth, local chicken, lysine, productivity.*

1. INTRODUCTION

Lysine is one of the limiting amino acids in broiler diet and it is used to calculate the remaining essential amino acids in the ration (Baker *et al.*, 2002). Lysine requirement of broilers is higher in low protein diets for maximum weight gain and feed efficiency (Labadan *et al.*, 2001). As widely described, increasing dietary lysine generally results in improved feed intake, feed conversion ratio and body weight gain (Sterling *et al.*, 2008). Moreover, lysine has impact not only on birds' growth but also on their ability against bird flu (H_5N_1) infection. Previously, Murray *et al.* (1998) found that addition of lysine at high levels to the diets can stimulate insulin secretion from pancreas by aggregating in plasma, which in turn releases amino and fatty acids from the bodily saved sources and leads to protein synthesis. Supportably, the report of Rubin *et al.* (2007) indicated that the addition and improvement of lysine in poultry diets improved birds' immune ability against different diseases.

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In Vietnam, Ac chicken is an indigenous breed with black of skin, meat and bones and is widely adopted in the Mekong Delta. Its meat quality is considered juicy with high levels of amino acids. Similar to other breeds, Ac chickens need amino acids for body growth and protein synthesis, of which lysine is the most important amino acid to concern. Previous work has focused on conservation (Thieu *et al.*, 2008), production (Phuong and Thien, 2008), protein and energy levels on performance and egg quality (Phuoc *et al.*, 2015) but little was known on the effects of lysine inclusion levels on Ac chicken. This study was therefore designed to determine growth performance, carcass composition and immunological response to influenza vaccination in Ac chickens supplemented with lysine at different content in the diet.

2. MATERIALS AND METHODS

2.1. Experimental design and diets

The experiment was conducted at Animal Research and Experiment farm, Tra Vinh University from Sep 2016 to Mar 2017.

The feeding experiment was allocated in a completely randomized design with 5 treatments and 3 replicates (thirty and sixteen birds for each treatment at 0-4 and 5-8 weeks of age, respectively). Vaccination against Newcastle disease was performed at the 5th day of age by nostril and eye drop method and bird flu at the 14th day of age by breast intramuscular injection. The diets were offered *ad libitum* and water was freely available throughout eight weeks. The experimental birds were fed with five diets including control (dietary lysine 1 and 0.9% of 0-4 and 5-8 week-old period, respectively) and other four diets were added with 5, 10, 15 and 20% lysine as compared with the control diet. Details of nutritional values and composition of diets of two phases are shown in Table 1, 2 and 3, respectively.

Table 1: Nutrient value (% of feed) of yellow corn and soybean meal

Feedstuffs	DM	ME, kcal/kg	CP	Lys	Met+Cys	Ca	P
Yellow corn	91.54	3,222	9.17	0.247	0.779	0.63	0.29
Soybean meal	90.96	2,500	44.96	1.289	1.967	0.63	0.67

Note, DM: dry matter; ME: metabolizable energy; CP: crude protein; Lys: lysine; Met+Cys: Methionine + Cystein.

Table 2. Experimental diets of birds in 0-4 week of age

Feedstuffs and nutrient value		Ctrl	Ctrl+5	Ctrl+10	Ctrl+15	Ctrl+20
Feedstuffs	Yellow corn, g	62.95	63.02	63.07	63.07	63.13
	Soybean meal, g	32.93	32.82	32.7	32.63	32.5
	Soybean oil, g	0.55	0.55	0.56	0.58	0.6
	HCl-Lysine, g	0.43	0.47	0.53	0.58	0.63
	DCP, g	1.95	1.95	1.95	1.95	1.95
	Shell, g	0.46	0.46	0.46	0.46	0.46
	Premix, g	0.25	0.25	0.25	0.25	0.25
	Salt, g	0.48	0.48	0.48	0.48	0.48
Nutrient values	ME, kcal/kg	2,900	2,900	2,900	2,900	2,900
	CP, %	21	21	21	21	21
	Lysine, %	1	1.05	1.1	1.15	1.2
	Ca, %	1.2	1.2	1.2	1.2	1.2
	P, %	0.78	0.78	0.78	0.78	0.78

Note, Ctrl: control treatment; Ctrl+5, Ctrl+10, Ctrl+15, and Ctrl+20: treatments adding lysine 5, 10, 15 and 20% compared with control treatment; DCP: Dicarbonate phosphorus; CP: crude protein; Ca: Calcium; P: Phosphorus.

Table 3. Experimental diets of birds in 5-8 week of age

Feedstuffs and nutrient values		Ctrl	Ctrl+5	Ctrl+10	Ctrl+15	Ctrl+20
Feedstuffs	Yellow corn, g	72.0	72.0	72.0	72.0	72.0
	Soybean meal, g	24.46	24.41	24.37	24.33	24.28
	HCl-Lysine, g	0.41	0.46	0.5	0.54	0.59
	DCP, g	2.1	2.1	2.1	2.1	2.1
	Shell, g	0.3	0.3	0.3	0.3	0.3
	Premix, g	0.25	0.25	0.25	0.25	0.25
	Salt, g	0.48	0.48	0.48	0.48	0.48
Nutrient values	ME, kcal/kg	2,930	2,930	2,929	2,928	2,926
	CP, %	18	18	18	18	18
	Lysine, %	0.9	0.95	0.99	1.03	1.08
	Ca, %	1.2	1.2	1.2	1.2	1.2
	P, %	0.78	0.78	0.78	0.78	0.78

2.2. Feed intake, body weight and carcass composition

Feed intake was recorded daily by weighing feed supply and refusal. Body weight was taken every week at 6 a.m. before chickens were fed. Feed conversion ratio was calculated as feed intake per unit of body weight. For carcass composition, 12 chickens (per treatment) were slaughtered at 8 weeks of age to determine the carcass, breast meat and leg meat ratios. Breast meat nutritional values (dry matter, crude protein, fat and ash content) were analyzed by Weende's proximate method (AOAC, 1990) and amino acid composition was determined using the high-pressure liquid chromatography. Internal organs such as spleen, thymus and bursa of Fabricius were separated and weighed and the relative organ weight was calculated as percentage of body weight.

2.3. Antibody response analysis

A total of 6 blood samples in each treatment were collected from 6 birds via their hearts to determine maternal antibody levels against the bird flu disease. Determining antibody against the bird flu disease was done using haemagglutination method (HA) titration as suggested by Thayer and Beard (1998).

2.4. Statistical analysis

Data on growth yield, bodyweight, carcass composition and immunological responses were analyzed by one-way of variance (ANOVA) in Minitab 13.2 (2000). The differences between experimental treatments were examined with Tukey's method.

3. RESULTS AND DICUSSION

3.1. Growth performance and feed utilization

Body weight gain (BWG) and feed conversion ratio (FCR) of birds were significantly influenced ($P<0.05$) by the different diets (Table 4), of which BWG was highest in the Ctrl+10 (52.4 g/bird/week) but was lowest in Ctrl (43.62 g/bird/week) diet. Similarly, the feed conversion of birds was most efficient in Ctrl+10 (2.09kg feed/kg weight gain), compared with other treatments. However, there was no significant difference regarding to feed intake of chickens among treatments. The present results partly supported the observations of Mehrdad (2012) and Faluyi *et al.* (2015) that lysine supplementation above NRC requirement (NRC, 1994) in broiler diets improved final body weight, weight gain and feed intake. Moreover, Corzo *et al.* (2005) found that lysine supplementation significantly improved the live body weight and feed conversion efficiency. Supportably, Usama *et al.* (2007) pointed out that the poor live weight performance of broilers fed with basal diets and the responses to supplemental L-lysine indicated that the basal diets were severely deficient in lysine. From the present work, it was also indicated that supplementary levels more than NRC requirement (>5%) was appropriate for enhancing growth rate of Ac chicken. This could be explained by the balance of amino acid content in the diets, where lysine is the basic unit to synthesize protein, it affects directly protein synthesis in the body of birds, and thereby improved growth rate of birds.

Table 4: Effects of dietary lysine on birds' body weight and feed conversion ratio

Variables	Ctrl	Ctrl+5	Ctrl+10	Ctrl+15	Ctrl+20	P
Initial BW, g/bird	21.3	22.0	21.0	22.6	21.6	ns
FI, g/bird/week	99.17±3.54	104.6±7.58	111.9±3.29	104.5±4.82	103.9±8.81	0.221
BWG, g/bird/week	43.62^{b}±1.62	48.0^{ab}±2.72	52.4^{a}±1.60	48.8^{ab}±2.29	48.1^{ab}±3.59	0.021
FCR	2.29^{a}±0.06	2.16^{b}±0.03	2.09^{b}±0.009	2.12^{b}±0.02	2.13^{b}±0.005	0.000

Note, FCR: feed conversion ratio; BW: bodyweight; BWG: bodyweight gain; FI: feed intake; ns: non-significant; ^{a,b} means with different superscripts within a row are significantly different at $P<0.05$.

3.2. Carcass composition and nutritional value of breast muscle

The composition of carcass and nutritional value of breast muscle of Ac chicken was not different among diets (P>0.05) (Table 5 and 6). The present output agreed with the finding of Tang *et al*. (2007), which stated that carcass weight and breast muscle yield were not affected by dietary lysine. Rostagno and Pack (1995) also reported that lysine had no effects on breast and leg muscle weight. Tesseraud *et al*. (2001) and Rezaei *et al*. (2004) showed that excessive lysine increased muscle weights and breast muscle weight. In term of nutritional value, dry matter and protein content of breast meat were similar to the report of Thieu *et al*. (2008). The results were consistent with findings of Khang and Ogle (2004), who concluded that crude protein and crude fat of thigh meat were not influenced by the replacement of duckweed for soybean and rice bran in chicken diet. Similar results were also found in amino acid composition, where no effect of lysine was observed.

Table 5: Effects of dietary lysine on birds' carcass performance

Variables	Ctrl	Ctrl+5	Ctrl+10	Ctrl+15	Ctrl+20	P
Body weight, g	395±17.2	400±17.0	397±18.6	399±19.6	404±19.6	0.941
Carcass, g	276±11.7	280±9.4	280±10.3	280±11.9	281±10.2	0.940
Carcass ratio, %	69.9±0.95	70.1±0.91	70.4±1.40	70.2±1.04	69.6±1.21	0.753
Breast muscle ratio, %	19.6±0.65	19.7±0.91	18.75±0.65	19.53±1.04	19.9±0.77	0.144
Thigh muscle ratio, %	20.5±0.68	20.3±1.0	20.4±1.0	20.9±0.58	20.1±0.83	0.511

Table 6: Effects of lysine on nutritional value and amino acid composition of breast muscle

Variables		Ctrl	Ctrl+5	Ctrl+10	Ctrl+15	Ctrl+20	P
Nutritional value	Dry matter (%)	25.75±1.13	25.76±0.42	26.03±0.66	26.06±1.11	25.22±0.89	0.488
	CP (%)	23.68±2.21	23.20±1.19	23.52±0.51	23.37±1.11	22.32±0.82	0.430
	Fat (%)	3.26±0.92	3.12±0.57	2.54±0.76	2.37±1.04	2.85±0.92	0.359
	Ash (%)	2.01±1.13	1.32±0.13	1.65±0.74	1.33±0.06	1.25±0.21	0.206
Amino acid (g/kg)	Aspartic	17.73±1.37	17.12±1.34	16.86±0.8	17.35±1.47	18.55±0.51	0.146
	Serine	17.48±0.71	18.84±5.17	16.28±0.68	17.02±1.71	17.3±1.12	0.525
	Glutamic	32.08±3.18	32.42±2.43	31.12±1.20	31.82±3.37	32.62±1.95	0.862
	Glycine	28.96±5.82	28.16±7.33	26.34±4.41	25.35±4.65	22.85±1.57	0.286
	Histidine	23.88±2.39	26.56±2.61	25.1±2.82	26.67±3.01	25.63±1.58	0.320
	Threonine	18.54±0.54	18.71±2.21	18.71±1.59	16.05±4.35	17.76±1.26	0.275
	Proline	15.22±1.76	14.34±1.15	14.57±1.48	14.7±1.45	14.05±1.01	0.675
	Cystine	9.10±1.60	7.08±2.04	9.27±1.94	9.97±1.34	9.45±1.55	0.067
	Tyrosine	27.22±3.03	27.93±2.01	25.76±2.54	28.21±4.43	26.83±5.87	0.818
	Valine	20.12±1.81	19.63±0.65	19.62±0.74	20.1±1.65	20.6±1.33	0.694
	Methionine	14.16±2.09	11.47±4.76	14.03±2.04	15.47±1.56	14.33±1.67	0.165
	Lysine	13.95±3.14	12.4±0.79	14.77±1.3	12.42±0.84	13.21±2.16	0.163
	Leucine	29.01±2.63	29.92±0.98	29.43±0.77	30.4±3.3	30.77±1.94	0.630
	Phenylalanine	30.57±3.87	32.64±2.11	29.95±2.78	32.88±4.7	30.99±1.82	0.448
	Tryptophane	2.13±0.13	2.05±0.04	2.14±0.09	2.05±0.15	1.98±0.08	0.072

3.3. Immunological responses to influenza vaccination

Evidences in the report of Kidd *et al.* (1997) and Konashi *et al.* (2000) showed that a dietary deficiency of lysine limited the synthesis of proteins and the proliferation of lymphocytes, as well as impaired immunological responses in birds, resulting in increases in morbidity and mortality in response to infection. There were also findings stating that an inadequate intake of dietary lysine reduced antibody responses and cell-mediated immunity in chickens (Chen *et al.*, 2003). In addition, lysine and methionine supplementation to the diet has improved the immunity of chickens against potential diseases (Rubin *et al.*, 2007). In the present study, there was no significant difference on birds' spleen ratio between diets; however, Fabricius and thymus ratio of birds was statistically influenced ($P<0.05$) by different amounts of dietary lysine (Table 7). In the Ctrl+20, Ctrl+15 and Ctrl+10, the ratios of Fabricius (0.146%, 0.141%, and 0.134%, respectively) and thymus (0.522%, 0.542%, and 0.536%, respectively) was higher than that of Ctrl with 0.104% of Fabricius and 0.382% of thymus. As dietary lysine contents increased from 1%:to 1.15% and from 0.9 to 1.03 in stages 0-4 and 5-8 weeks of age, respectively, the ratio of Fabricius and thymus gradually increased. Lysine is an essential amino acid that is necessary to produce proteins including antibodies; thus, adequate dietary levels of lysine is needed to support optimum efficacy of the immune system. Although the value of $\log_2$ HI titre of the Ctrl treatment tended to be lower than those of the other treatments, the antibody titre against avian influenza at 28 and 56 day-old were not significantly different among treatments. Mehrdad (2012) reported that increased lysine content in diets of broilers in excess of NRC recommendation could improve immune system functions against Newcastle disease. Therefore, an appropriate vaccination regime should be taken into consideration for Ac chicken to fully achieve the efficacy of immune function.

Table 7: Effects of lysine on birds' immune responses

Variables	Ctrl	Ctrl+5	Ctrl+10	Ctrl+15	Ctrl+20	P
Spleen ratio, %	0.09±0.013	0.10±0.023	0.105±0.023	0.104±0.02	0.103±0.019	0.815
Fabricius ratio, %	0.104^{b}±0.02	0.123^{ab}±0.01	0.134^{a}±0.01	0.141^{a}±0.02	0.146^{a}±0.02	0.000
Thymus ratio, %	0.382^{b}±0.07	0.475^{ab}±0.03	0.536^{a}±0.08	0.542^{a}±0.06	0.522^{a}±0.06	0.001
H_5N_1 antibody, 28d*	6.81±1.05	7.5±0.75	7.64±0.63	7.08±1.38	7.28±0.85	0.838
H_5N_1 antibody, 56d*	6.75±0.90	7.72±0.25	7.5±0.43	7.33±0.76	7.18±0.71	0.482

*Note, Ctrl: control treatment; Ctrl+5, Ctrl+10, Ctrl+15, and Ctrl+20: treatments adding 5, 10, 15, and 20% lysine compared with control treatment; *: $\log_2$ HI titre; ^{a,b} means with different superscripts within a row are significantly different at $P<0.05$.*

4. CONCLUSION

The present study demonstrated that dietary lysine supplementation at 10% improved growth performance and feed conversion ratio but carcass composition were not influenced. The ratios of spleen and thymus were improved but immunological responses to influenza vaccination remained unchanged despite the increased lysine content in the diet.

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