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Full Length Research Paper

Effect of dietary lysine on performance and immunity parameters of male and female Japanese quails

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The present study was conducted to investigate the effects of dietary lysine (Lys) and sex on growing quail performance and immunity parameters. The six dietary Lys: 10, 11.5, 13.0, 14.5, 16.0 and 17.5 g/kg were provided. This experiment was carried out in a completely randomized design arrangement from 3 to 24 days. Each treatment was consisted of 5 floor pens (50 quail chicks). Body weight (BW) and body weight gain (BWG) increased significantly (P<0.05) with dietary Lys supplementation up to 14.5 g/kg. The quails that consumed 14.5 g/kg Lys had the best feed conversion ratio (FCR). The carcass weight, breast weight and yield, thigh weight and yield, mortality and response to sheep red blood cell (SRBC) were significantly (P<0.05) affected by dietary Lys. The breast yield, thigh weight and yield, white blood cells count, monocyte percentage and response to SRBC significantly (P<0.05) were influenced by sex. Neither age nor sex had any significant effect on lymphocyte percentage, heterophile percentage and heterophile to lymphocyte ratio at 24 days of age. In this study, an interaction of sex with Lys were not significant (P>0.05) for any traits. It concluded that during the 3 to 24 days of age optimum BW, BWG, FCR and immunity status could be obtained with 14.5 g/kg Lys, so these levels is more than NRC 1994 recommendation.

Key words: Lysine, Japanese quail, performance, immunity.

INTRODUCTION

Japanese quail (*Coturnix coturnix Japonica*) belongs to the order Galliformes and the family Phasianidae like the chicken (Karaalp, 2009). The Japanese quail possesses several advantages, such as rapid growth, early sexual maturity, high rate of egg production, and a short generation interval (Mandel et al., 2006).

Essential amino acid recommendations by NRC 1994 for Japanese quail are largely based on researches that conducted at least 5 to 6 years before 1994's but, the meat production performance of Japanese quails has also been improved during recent years due to genetic selection. Therefore, there is need of updating optimal nutritional requirements of Japanese quails with the improvement in genetic makeup to exploit production potentiality (Kaur et al., 2008).

Lysine (Lys) is the second limiting amino acid after methionine in a maize–soybean based diet and most scientists use Lys as the basis to which all other amino acids are related when generating an "ideal balance", furthermore lysine and sulfur amino acids (SAA) are known to exhibit specific effects on carcass composition (Corzo et al., 2002).

In chickens as well as in mammals, it has been shown that deficiency or excess of dietary amino acids (Augspurger and Baker, 2007; Dozier et al., 2008) alters performance and immune responses but information on the effects of lysine on performance and immunity of Japanese quails is scanty (Kaur et al., 2008). The literature is also silent on the amino acid values in different feedstuffs and their requirements for Japanese quails. Therefore, the present study was conducted to elucidate the response of growing Japanese quail to different levels of Lys for optimum growth performance, feed utilization efficiency and immunity.

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Table 1. Ingredients and calculated composition of experimental diets (percentage as-fed basis).

Item	Graded level of lysine (g/kg)					
	10.0	**11.5**	**13.0**	**14.5**	**16.0**	**17.5**
Ingredient (%)						
Ground corn	52.34	52.34	52.34	52.34	52.34	52.34
Soybean meal(44% cp)	26.24	26.24	26.24	26.24	26.24	26.24
Corn gluten meal	15.16	15.16	15.16	15.16	15.16	15.16
Calcium carbonate	1.36	1.36	1.36	1.36	1.36	1.36
Dicalcium phosphate	0.88	0.88	0.88	0.88	0.88	0.88
Sodium chloride	0.16	0.16	0.16	0.16	0.16	0.16
Sodium bicarbonate	0.19	0.19	0.19	0.19	0.19	0.19
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral mineral[2]	0.25	0.25	0.25	0.25	0.25	0.25
L-Lys·HCl	0	0.19	0.39	0.59	0.80	1.00
Gln	0.75	0.60	0.45	0.30	0.15	0
L-Thr	0.17	0.17	0.17	0.17	0.17	0.17
DL-Met	0.07	0.07	0.07	0.07	0.07	0.07
Inert filler[3]	2.18	2.14	2.09	2.04	1.98	1.93
Total	100	100	100	100	100	100
Calculated composition						
AME (kcal/kg)	2900	2900	2900	2900	2900	2900
CP (%)	24	24	24	24	24	24
Lys (%)	1.00	1.15	1.3	1.45	1.6	1.75
Met (%)	0.55	0.55	0.55	0.55	0.55	0.55
Met + Cys (%)	0.82	0.82	0.82	0.82	0.82	0.82
Thr (%)	1.12	1.12	1.12	1.12	1.12	1.12
Nonphytate P (%)	0.3	0.3	0.3	0.3	0.3	0.3
Calcium (%)	0.8	0.8	0.8	0.8	0.8	0.8
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15

[1]Vitamin premix include per kilogram of diet: vitamin A (vitamin A acetate), 3600 IU; cholecalciferol (D3), 800 ICU; vitamin E (DL-α-tocopheryl acetate) , 7.7 IU; menadione, 0.8 mg; vitamin B12 0.01 mg; folic acid, 0.4 mg; choline chloride, 170 mg; D-pantothenic acid, 12 mg; riboflavin, 2.6 mg; niacin, 4 mg; biotin, 0.2 mg; thiamin, 0.7 mg; pyridoxine. [2]Mg; butylated hydroxytoluene, 125 mg. Mineral premix supplied the following per kilogram of diet: manganese, 16 mg; zinc, 15 mg; iron, 8 mg; copper, 4 mg; iodine, 1.6 mg; selenium, 0.08 mg; butylated hydroxytoluene, 125 mg. [3]Filler represents inert space (builder' s sand) in the diet to which L-Lys-HCl was added to derive the projected Lys level.

MATERIALS AND METHODS

Birds

Day-old Japanese quail chicks (*C. cotornix Japonica*) were provided from commercial hatchery and reared on a common starter diet during the first 3 days of age. At 3 days of age, chicks were weighed individually and based on similar body weight, 1500 chicks selected and randomly allocated to 30 floor pens (50 chicks per pen) so average initial body weight and variance were similar between all pens. Each of the experimental diet was then randomly assigned to 5 pens.

All birds reared on floor pens (80 × 150 × 100 cm) on wood shavings in temperature-controlled house during 3 to 24 days of age. The temperature was 35ºC in the first week and reduced by 2.5ºC/week up to 3 weeks of age. The birds received 24 h of light/day during the 24 days of age. Food and water were available *ad libitum*. All chicks received feeds from placement until 24 days of age in mash form, according to its treatment.

Diets

A basal diet was deficient in lysine content (1%) and formulated mainly based on corn, corn gluten meal, and soybean meal (Table 1). Crystalline amino acids were included to assure the minimum levels of all other essential amino acids in a manner that would meet or exceed NRC (1994) recommendations (Corzo et al., 2008). The calculated content of AME was 2,900 kcal/kg (12.1 MJ/kg), and the analyzed concentrations of CP and lysine were 261 and 11 g/kg, respectively. The lysine level was gradually increased in 6 further isonitrogenous diets by stepwise inclusion of L-lysine •HCl at the expense of L-glutamine to achieve the following lysine concentrations (g/kg): 10, 11.5, 13.0, 14.5, 16.0 and 17.5 (Table 1). L-lysine•HCl, DL-methionine and L-threonine were feed grade quality. All ingredients with the exception of the variable ones (L-glutamine, L-lysine•HCl and inert filler) were mixed as a single lot and the mix were divided into 6 parts. L- Glutamine, L-lysine •HCl and inert filler were added separately in the respective proportions to each diet then mixed again.

Table 2. Dietary Lys effect on mixed-Japanese quails' performance at 3 to 24 d of age[1].

Variable	Dietary lysine (g/kg)						SEM[2]	CV[3]	P-value
	10.0	11.5	13.0	14.5	16.0	17.5			
Initial body weight	17.76	17.62	17.68	17.56	17.53	17.64	0.42	2.38	0.970
Body weight									
BW, 10 days (g)	27.91^{b}	28.07^{b}	28. 76^{b}	31.56^{a}	31.15^{a}	30.16^{ab}	1.46	4.95	0.0006
BW, 17 days (g)	50.96^{c}	56.96^{b}	57.25^{b}	62.22^{a}	60.15^{ab}	61.88^{a}	2.78	4.77	0.0002
BW, 24 days (g)	93.83^{c}	97.64^{c}	100.01^{bc}	110.62^{a}	107.48^{ab}	110.51^{a}	5.12	4.96	0.0005
Body weight gain									
BWG, 3-10 days (g)	10.23^{c}	10.56^{bc}	11.13^{bc}	13.99^{a}	13.61^{a}	12.52^{ab}	1.29	10.75	0.001
BWG,10-17 days (g)	23.05^{c}	26.89^{b}	27.49^{b}	30.66^{ab}	29.00^{ab}	31.71^{a}	2.35	8.38	0.001
BWG,17-24 days (g)	39.86^{b}	42.76^{ab}	46.67^{ab}	48.40^{a}	47.32^{a}	48.63^{a}	4.54	9.95	0.076
BWG, 3-24 days (g)	72.27^{c}	81.83^{b}	84.13^{ab}	93.06^{a}	89.94^{ab}	92.87^{a}	5.84	6.82	0.0005

[1]Means with different superscripts within the same row differ significantly (P<0.05). [2]Standard error of means. [3]Coefficient of variation.

Measurements

Performance parameters

The body weight, feed consumption of all birds were recorded at 3, 10, 17, 24 day of age, the average body weight gain was calculated weekly. The mortality was recorded daily. Feed conversion was corrected for mortality and represents grams of feed consumed by all birds in a pen divided by grams of body weight gain per pen (Fisher, 1998).

At 24 days of age, 10 female and 10 male quails from each treatment were selected and weighed individually before slaughter. The quails were starved for 4 h, but drinking water was supplied. The quails were slaughtered by severing the jugular vein, blood samples were collected into 1.5 ml tubes immediately thereafter.

The quails were plucked mechanically after hot water scalding then carcasses was eviscerated by hand. Weights were individually recorded for the carcass, thigh, breast, liver and total alimentary tract. The yields of different carcass traits were expressed as percentage to final body weight (Fatufe et al., 2004).

Blood parameters

At 21 days of age blood samples were collected from two birds in each pen into the heparinzed tubes from the jugular vein. The samples were transferred to laboratory immediately then blood erythrocyte count, blood leukocyte count, differential leukocyte count; lymphocyte (Lym), monocyte (Mon) and heterophile (Het) was measured.

At 24 days of age, four quails from each pen (2 from each sex) were bled to collect serum. After overnight clotting at 4℃, the samples were centrifuged for 20 min at 4,000 × g then blood serum was frozen until analysis.

Immunity response parameters

At 14 days of age, 2 female and 2 male quails were selected from each pen (that is, 20 birds/dietary treatment providing 100 birds in all) and were inoculated intramuscularly with 0.1 ml of a 0.5% suspension of sheep red blood cell (SRBC). For measuring the primary immunity response blood samples were obtained from all SRBC injected birds at 0 and 5 days post-inoculation.

All the samples were incubated at 37ºC for one hour to aid clotting and retraction then centrifuged at 15000 ×g for 5 min for collection of sera. Blood serum was frozen until analysis for antibody titers could be performed. The all serum samples after thawing were used for analysis. All the microtitre plates (U-bottomed) were rinsed with phosphate-buffered saline (PBS; pH 7 to 6) then dried before the haemagglutination antibody (HA) titre was estimated by a micro-haemagglutination method using twofold serial dilutions of sera (Biswas et al., 2006).

Statistical analysis

Data in this experiment were evaluated by analysis of variances (ANOVA) in a completely randomized design. Pen was used as the experimental unit for analysis. All data were examined for normality distribution for ANOVA analysis was then analyzed by the general linear models (GLM) procedure of SAS (1996). Lysine and sex effects (P<0.05) were separated using the Duncan multiple range test option of SAS (1996) with an alpha (α) of 0.05.

RESULTS AND DISCUSSION

Performance

The performance results are presented in Tables 2 and 3. There was no significant difference (P>0.05) in initial body weight, between experimental treatments. Dietary Lys significantly (P<0.05) affected BW at 10, 17 and 24 days of age. BW was increased by Lys increasing, so the quails that consumed at least 14.5 g/kg Lys was heavier than other Lys treatments and beyond this level there was no significant difference in BW between treatments.

Body weight gain (BWG) within 3 to 10, 10 to 17, 17 to 24 and 3 to 24 days was significantly (P<0.05) affected by dietary Lys. BWG was in line with BW so the quails that consumed at least 14.5 g/kg Lys had highest BWG than others. The BWG of quails that consumed dietary Lys more than NRC recommendation had significant

Table 3. Dietary Lys effect on mixed-Japanese quail feed intake and feed conversion at 3-24 d of age[1].

Variable	Dietary lysine (g/kg)						SEM[2]	CV[3]	P-value
	10.0	11.5	13.0	14.5	16.0	17.5			
Daily feed intake									
FI, 3-10 days (g)	5.36	5.97	5.58	5.55	5.58	6.20	0.54	9.53	0.293
FI, 10-17 days (g)	11.44	12.02	11.12	11.59	10.51	11.96	1.11	9.7	0.423
FI, 17-24 days (g)	25.47^{a}	25.66^{a}	24.56^{ab}	23.25^{bc}	23.34^{bc}	22.20^{c}	1.27	5.31	0.007
FI, 3-24 days (g)	42.27^{ab}	43.66^{a}	41.28^{ab}	40.39^{b}	40.37^{b}	39.43^{b}	1.88	4.53	0.059
Feed conversion ratio									
FCR, 3-10 days (g:g)	3.15^{a}	3.22^{a}	3.30^{a}	2.38^{b}	2.46^{b}	2.99^{a}	0.296	10.14	0.0007
FCR, 10-17 d (g:g)	2.66^{ab}	2.55^{abc}	2.89^{a}	2.27^{bc}	2.18^{c}	2.27^{bc}	0.252	10.21	0.0056
FCR, 17-24 days (g:g)	3.89^{a}	3.66^{ab}	3.17^{bc}	2.97^{c}	2.89^{c}	2.75^{c}	0.399	12.38	0.0039
FCR, 3-24 days (g:g)	3.23^{a}	3.14^{a}	3.12^{a}	2.51^{b}	2.54^{b}	2.67^{b}	0.15	5.24	<0.0001

[1]Means with different superscripts within the same row differ significantly (P<0.05). [2]Standard error of means. [3]Coefficient of variation.

Table 4. Dietary Lys and sex effects on slaughter related traits and mortality of quails at 24 days of age[1].

Variable	Dietary lysine (g/kg)						Sex		Effects			CV[2]	SEM[3]
	10.0	11.5	13.0	14.5	16.0	17.5	Female	Male	T	S	T×S		
Carcass weight (g)	89.89^{c}	85.01^{bc}	96.47^{abc}	103.13^{a}	102.92^{a}	99.25^{ab}	98.75	96.73	*	NS	NS	6.96	6.80
Breast weight (g)	26.26^{c}	28.24^{bc}	29.66^{ab}	30.42^{ab}	31.51^{a}	30.65^{ab}	29.27	29.64	*	NS	NS	8.51	2.50
Breast yield (%)	23.14^{c}	23.45^{bc}	24.84^{abc}	24.49^{abc}	25.99^{a}	25.20^{ab}	23.93^{b}	25.11^{a}	*	*	NS	7.37	1.80
Tight weight (g)	16.45^{b}	18.28^{ab}	18.23^{ab}	18.72^{a}	19.74^{a}	19.52^{a}	17.57^{b}	19.41^{a}	*	*	NS	9.62	1.77
Tight yield (%)	14.44^{c}	15.12^{bc}	15.22^{bc}	14.99^{bc}	16.32^{a}	15.99^{ab}	14.29^{b}	16.40^{a}	*	*	NS	6.55	1.00
Liver weight (g)	3.47	3.75	3.42	3.47	3.56	3.38	3.52	3.50	NS	NS	NS	12.10	0.42
Liver yield (%)	3.06	3.92	2.86	2.78	2.95	2.79	2.89	2.97	NS	NS	NS	10.24	0.30
GI weight (g)	14.48	14.79	14.23	14.89	14.43	14.11	14.61	14.36	NS	NS	NS	11.29	1.63
GI yield (%)	12.76	12.43	11.93	11.92	11.99	11.64	12.01	12.21	NS	NS	NS	10.57	1.28
Mortality (%)	3.2^{a}	2.5^{a}	1.9^{ab}	1.4^{b}	0.98^{b}	1.3^{b}	2.43	2.86	*	NS	NS	14.56	0.45

[1]Means with different superscripts within the same row differ significantly (P<0.05). [2]Coefficient of variation. [3]Standard error of means.

(P<0.05) difference with other lower levels. This findings was agree with Dozier et al. (2008) in broilers, who concluded that the NRC (1994) Lys requirement of 0.85% (total basis) is not adequate from 6 to 8 weeks of age for Ross × Ross 708 broilers.

There were no significant differences (P>0.05) in feed intake at 3 to 10 d, 10 to 17 days but Lys significantly (P<0.05) affected feed intake at 17 to 24 and 3 to 24 days. The significant difference in feed intake at 17 to 24 day may occurred for Lys deficiency diets, in the other hand the quails with inadequate Lys intake consumed more feed than other treatments for compensating the deficient Lys uptake.

Dietary lysine significantly (P<0.05) affected feed conversion ratio (FCR) at 3 to 10, 10 to 17, 17 to 24 and 3 to 24 days of age (Table 3). With increasing dietary Lys supplementation FCR showed significantly (P<0.05) improvement. However, the quails that consumed 14.5 g/kg dietary Lys showed the best FCR in compared with the other dietary Lys treatments.

Some studies reported that the Lys has positive effects on broiler body weight gain of increasing dietary Lys is obtained through the improvement of both feed intake and feed conversion (Fatufe et al., 2004; Sterling et al., 2005; Tesseraud et al., 2009) whereas other reported that increasing Lys only improved feed conversion without affecting feed intake (Corzo et al., 2002; Corzo et al., 2003). In the present study with Japanese quails we found that effect of Lys on body weight by means of affecting on the both feed intake and feed conversion.

Carcass constitutes

The effects of dietary Lys and sex on carcass constitutes at 24 days is presented in Table 4. Dietary Lys significantly (P<0.05) affects carcass weight, breast weight and yield, thigh weight and yield. Carcass weight increased up to 103.13 g with 14.5 g/kg dietary Lys although this Lys level has no significant difference with 13 g/kg Lys.

Table 5. Dietary Lys and sex effects on hematology and immunity parameters of Japanese quails at 24 days of age[1]

Variable	Dietary lysine (g/kg)						Sex		Effects			CV[2]	SEM[3]
	10	11.5	13	14.5	16	17.5	Female	Male	T	S	T×S		
Packed cell volume (%)	33.87	33.48	34.51	36.41	33.61	35.26	31.54[b]	37.51[a]	NS	*	NS	10.85	3.76
Red blood cell (n×10[6]/µl)	2.97[b]	3.08[ab]	3.12[ab]	3.30[ab]	3.11[ab]	3.41[a]	3.14	3.19	*	NS	NS	9.28	0.29
White blood cell (n/µl)	23863	20013	22100	24538	23050	21075	20179[b]	24700[a]	NS	*	NS	20.33	4563
Differential leucocytes count													
Lymphocyte (%)	72.00	70.62	73.00	74.87	72.62	75.62	72.12	74.12	NS	NS	NS	6.36	4.65
Monocyte (%)	3.37	2.75	2.75	2.87	2.62	2.37	2.37[b]	3.20[a]	NS	*	NS	45.03	1.32
Heterophil (%)	20.25	20.12	21.62	22.12	21.12	19.00	19.91	21.50	NS	NS	NS	20.26	4.19
Hetro/Lympho ratio	0.280	0.273	0.296	0.311	0.296	0.251	0.290	0.279	NS	NS	NS	27.2	0.07
Humeral immunity													
Response to SRBC	5.62[b]	7.25[ab]	7.37[ab]	8.12[a]	7.62[a]	6.87[ab]	7.16	7.12	*	NS	NS	23.17	1.65

[1]Means with different superscripts within the same row differ significantly (P<0.05). [2]Coefficient of variation. [3]Standard error of means.

The breast weight and yield showed the best values between 13 to 16 g/kg dietary Lys level. The quails that consumed 16 g/kg dietary Lys had the highest amount of thigh weight and yield. This result was agree with other finding in broiler or turkey, that concluded Lys need is higher when breast and thigh weight was included as response of requirement determination (Coleman et al., 2003; Corzo et al., 2002; Fatufe et al., 2004; Kaur et al., 2008; Wang et al., 2006).

Sex had no significant (P>0.05) effect on the carcass and, breast weights but breast yield, thigh weight and yield were significantly (P<0.05) affected by sex, so in all mentioned traits male quails presented the higher amount than female quails. Neither Dietary Lys nor sex had any significant (P>0.05) effect on liver, gastrointestinal tract weights and yields.

Supplementation of diets with Lys significantly (P<0.05) reduced mortality during 3 to 24 d of age, so quails, which consumed diet with 16 g/kg Lys content, represented minimum mortality in compared with other treatments. Mortality of quails during 3 to 24 days of age did not differ significantly (P>0.05) between male and female quails.

Blood parameters

The effects of dietary Lys and sex on hematology parameters at 24 days were presented in Table 5. Dietary Lys had no significant (P>0.05) effect on packed cell volume (PCV) at 24 days of age, but packed cell volume was differed significantly (P<0.05) between male and female quails, so male quails had more PCV than female ones. The sex has no significant (P>0.05) effect on red blood cell (RBC), but Lys had significant (P<0.05) effect on red blood cell, it was increased numerically with increasing dietary Lys level but to our knowledge, there are no similar reports in the literature on Japanese quails for comparing our results.

Immunity response

The effects of dietary Lys and sex on white blood cell (WBC) count, differential leukocyte count; lymphocyte (Lym), monocyte (Mon), heterophile (Het) and humoral immunity (response to SRBC injection) were shown in Table 5. Lys had no significant (P>0.05) effect on WBC, but sex had significant (P<0.05) effect on WBC so the male quails (24700 n/µl) had higher WBC than the female quails (20179 n/µl).

Neither Lys nor sex had any significant (P>0.05) effect on blood lymphocyte, heterophile and heterophile to lymphocyte ratio at 24 days of age. Sex has significant (P<0.05) effect on blood monocyte so the male quails (3.2%) had more monocyte than the female quails (2.37%). Lys had significant (P<0.05) effect on response to SRBC injection as humoral immunity of quails but sex had no significant (P>0.05) influence on response to SRBC.

Our finding indicated that dietary supplementation of Lys improved humoral immunity of growing quails at 24 days of age so the quails who consumed diet with 14.5 g/kg dietary level showed the highest amount of immunity response, these finding was agree with (Biswas et al., 2006; Scholtz et al., 2009) who concluded that SRBC injection could use in growing quail as immunity response and nutrient supplementation has a beneficial effect on immune responses in growing Japanese quail.

Conclusion

This study indicated that a better response can be obtained with 14.5 g/kg dietary Lys, when body weight, feed conversion ratio were considered and may be at least 16.0 g/kg when breast and thigh meat properties are taken into account in Japanese quail from 3 to 24 d of age, so these levels are more than NRC (1994) recommendation for Japanese quail.

Furthermore, humeral immune system functions of healthy quails were improved numerically by fortifying diets with Lys in excess of NRC (1994) recommendations, although it did not differ statistically with NRC 1994 recommendation level. Future research, however, should compare dietary Lys effects on immune system functions of quails reared in conventional environments or during an infectious challenge.

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