

## EFFECT OF DIETARY PROTEIN AND LYSINE LEVELS ON GROWTH PERFORMANCE AND IMMUNE RESPONSE IN BROILER CHICKS

A. M., Orma; T. I., Mohamed & Basma, M. Gouda

Dept. of Nutrition & Nutritional Deficiency Diseases, Faculty of Vet. Med., Mansoura University

### ABSTRACT

The present work was carried out to study the effect of decreasing the dietary protein content by 10% (LCP-diet) with supplementation of lysine (0, 0.2, and 0.4%) over the recommended level in broiler diets on growth performance, carcass traits, blood metabolites, immune response. One day old Cobb chicks (120) were used. The chicks were distributed randomly into 4 groups and fed 23% or 20.7% CP for the starter diets, 20 or 18.2% CP for the grower and 18 or 16.2 % CP for the finisher diets with different dietary lysine level (1.1, 1.29 or 1.48%) for starter diets, 1, 1.22 or 1.4% for the grower diet and 0.85, 1.05 or 1.25% for the finisher diets, respectively and 3200 Kcal ME/Kg. The chicks were vaccinated against Avian Influenza, Newcastle and Gumboro diseases at proper time. The growth performance indices (body weight, feed intake and feed conversion ratio) were measured. Blood samples were collected from the broilers at 14, 21 and 28 days of age without anticoagulant for separation of sera to detect titer of antibodies against Newcastle disease vaccine using haemagglutination inhibition test (HI) as indicative of birds immune response in the different experimental groups and also at 42 days of age, blood samples collected for determination of serum metabolites. At the end of the experiment, 5 chicks from each group were slaughtered to obtain some of the carcass traits.

The results revealed that body weight and feed conversion ratio of the broiler fed 10% LCP-diet supplemented with 0.4% lysine above that recommended for the broilers significantly improved on comparison to those fed the LCP diets supplemented with 0, 0.2% lysine. Feeding the broiler chicks the LCP-diet decreased the body weight gain and increased the feed conversion ratio. The abdominal fat % in the carcass of the broiler chicks fed LCP-diet supplemented with 0.4% lysine were lower than those fed on the diets supplemented with 0, 0.2% lysine. Feeding LCP-diet supplemented with 0.4% lysine increased serum total protein, albumin, and globulin and decreased serum total lipids, cholesterol, triglycerides, low density lipoprotein and high density lipoprotein.

It can be concluded that feeding the broiler chicks on LCP-diet supplemented with 0.4% lysine diet resulted in better growth performance, lower total lipids, cholesterol,

*triglycerides, low density lipoprotein and high density lipoprotein and higher total protein, albumin, globulin in comparing to those fed 0 or 0.2% lysine supplemented diet. Supplementing the low protein diet with 0.4% lysine improved the HI titer in the broilers vaccinated against NDV at 14, 21 and 28 days of age.*

### INTRODUCTION

Malnutrition has been identified in many countries particularly in the developing countries. Majority of malnourished people live in Asia and Africa (Iwe and Onadipe, 2001), thus negatively impacting on the physical and health condition of its people. Poultry production is one major mean of solving part of this problem (Nworgu et al., 2000). Feeding poultry presents a great challenge to producers and nutritionists in several tropical countries as meeting the nutritional requirements for growing birds constitutes the majority of costs associated with poultry production (May et al., 1996), and certainly is becoming an issue of even greater significance as the prices of feed ingredients continue to rise. A large portion of that cost involves in meeting the amino acid requirements of the birds (Corzo et al., 2004; Elts et al., 2005).

The amino acid pattern provided in diets must exactly matches the birds amino acid requirements, excesses can be avoided consequently; protein accretion can occur with maximum efficiency, excess dietary protein increased heat production and water consumption which increased moisture content of litter (Alleman and Leclercq, 1997). Lowering crude protein in the diet could reduce the feed costs and decreased nitrogen excretion (Nahm, 2002 and Namroud et al., 2008), and allowed for using of a greater variety of feed stuffs (Kidd et al., 1996), which can be valuable in itself as a method to in-

crease flexibility in the choice of locally available feed stuffs and potentially decreasing fluctuation in costs. Dietary protein level therefore, has major effect on growth performance and overall cost of finished product. So using of LCP-diet supplemented with amino acid for broiler feeding has been received greater interests in the recent years in order to reduced feeding costs and environmental pollution (Namroud et al., 2008). The use of synthetic amino acid to meet the amino acid needs of broilers could result in production of cost effective diets.

In chickens as well as mammals, it has been shown that deficiency or excess of dietary protein (Payne et al., 1990) or amino acids (Tsalagbe et al. 1987) altered immune responses. The magnitude of an immune response and subsequent alterations in nutrient metabolism is dependent upon a nutritionally complete diet (Webel et al., 1998). The efficiency of dietary protein is primarily dependent upon an adequacy of lysine and methionine (most limited amino acids). So, appropriate supplementation of ration deficient in these amino acids markedly improved their protein quality (Ichhponani et al., 1981). With progress in biotechnology, the cost of production of each amino acid particularly lysine has been significantly reduced, which has been one of the key factors in the expansion of use of amino acids in animal feed. Therefore, the objective of this study is to further evaluate the probability of lowering

the dietary protein content with lysine supplementation and its effect on growth performance, dressed carcass, serum metabolites and immunocompetence in broiler chicks.

## **MATERIALS AND METHODS**

### **Birds, diets and management**

A total of 180 one day old Cobb chicks obtained from a commercial supplier were randomly distributed into 4 equal triplicate groups (15 chicks / replicate) and raised in a floor pen bedded with wheat straw to maintain a good litter. The pen was supplied with feeders, waterers and heaters. The chicks were tagged using wing number tags. The chicks were fed the respective experimental diets according to the suggested design. The diets were formulated to maintain the requirements of energy (3200 kcal ME/kg) with decreasing protein by 10% than that recommended by **NRC (1994)** and supplementation with lysine by (0, 0.2 & 0.4% of the diet) over the recommended. The diets crude protein of the diets was analyzed according to AOAC (1990). The composition of the experimental diets is shown in table 1.

The broiler chicks were reared during the period from mid of February to first of April, 2009. During the first 2 weeks of chicks' age the brooding temperature was maintained at 35-32°C, and then the temperature was gradually lowered till reach to 2°C by the beginning of the third week of age. The pen was naturally ventilated and electric light was continuously maintained (24 hours/day). The diets and water were available ad libitum throughout the experimental period (6 weeks). The chicks were vaccinated against avian influenza by subcutaneous injection and

Newcastle disease at 8<sup>th</sup> day of age by HitchnerB1 and at 18<sup>th</sup>, 28<sup>th</sup> days of age by lasota vaccine in drinking water and against Gumboro at 10<sup>th</sup> and 20<sup>th</sup> days of age in drinking water.

### **Indices of broiler performance, dressed carcass and abdominal fat**

The chicks were weighed at one day old to obtain the average initial body weight then weighed every week and the average body weights for the chicks in each group were weekly calculated. The average feed intake per chick throughout the experimental period (6w) for each group was recorded and feed conversion ratio was calculated. At the end of the experiment, five birds from each group were randomly taken and fasted for 12 hours, weighed, slaughtered to complete bleeding, dressed and eviscerated for determination of the dressed carcass % and abdominal fat weight and percent in relation to live body weight.

### **Blood sampling, serum metabolite and immune response**

Blood samples were collected from 5 chicks from each group at 42 days of age via wing vein puncture. Sera were obtained by allowing blood samples to coagulate then centrifuged at 3000 rpm for 15 minutes. The separated sera were frozen at -20°C in a deep freeze until used for determination of glucose (**Trinder and Ann, 1969**), total lipid (**Zollner and Kirsch, 1962**), triglycerides (**Fassati and Precipe, 1962**), cholesterol (**Allain et al., 1974**), high density lipoproteins (**Burstein et al., 1970**), low density lipoproteins (**Friede Wald et al., 1972**) total protein (**Goarnal et al., 1949**) and albumin (**Doumas et al., 1971**)

using already prepared analyzing chemical kits and after the instructions of the producer (GOD-PAP, BIODIAGNOSTIC and STANBIO). Globulin concentration was calculated as the difference between total protein and albumin.

Blood samples were collected from 5 chicks of each group at 14, 21 and 28 days of age without anticoagulant for separation of sera which were used to detect titer of antibodies against Newcastle disease vaccine using haemagglutination inhibition test (HI) as indicative of bird's immune response.

#### **Statistical analysis :**

Data obtained in the present work were statistically analyzed for analysis of variance (ANOVA) and least significant difference (LSD) as described by **Snedecor and Cochran (1967)**.

## **RESULTS AND DISCUSSION**

### **Body weight, body weight gain, feed intake, feed conversion ratio and dressed carcass :**

The data concerned with body weight development (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) showed that decreasing the dietary protein level by 10% than that of the **NRC (1994)** recommendation levels significantly decreased BW, BWG and significantly increased the values of FCR of broiler chicks even when lysine is supplemented by 0.2% above that recommended by **NRC (1994)**. However, increasing the level of lysine supplementation to 0.4 % levels significantly improved the BW, BWG and FCR recorded at 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks of the experiment (Table 2). **Alctor et al. (2000)** found that supplementing the diet

with amino acids improved growth of broilers. Also, **Sterling and Pestl (2003)** demonstrated that different levels of supplemental lysine increased BWG of broiler chicks from 0 to 21 days of age. The improvement in BW of broiler chicks fed LCP-diets supplemented with L-lysine indicated that lysine supplementation improved protein utilization (**El-Sherbiny et al., 1997 and Khalifa, 2001**). With this concept, **Abdallah et al. (2005)** found that BWG and FCR of broiler chicks fed LCP-diets (20 to 18% CP from 0 to 3 wk and 18 to 16% CP from 4 to 7 wk of age) supplemented with methionine and lysine was not significantly different from those fed the 23 to 20% CP-diet. Similarly, **Khan et al. (2010)** found that reduction of protein level from 23% to 19% had no detrimental effects on BWG when supplemented with lysine. The improvement in WG with LCP-diet supplemented with EAA could be due to reduced heat increment, which was associated with the metabolism of excess protein. Reduced heat increment led to reduced heat stress and therefore, improved WG.

The effect of decreasing the dietary crude protein level by 10% in the present study resulted in high feed intake and FCR whatever the diet supplemented with excess lysine or not (Table 3). The recorded FCR are 1.89 for the control diet compared to 2.54, 2.42 and 2.28 for the LCP-diet supplemented with 0, 0.2% or 0.4% lysine diets, respectively. **Nascimento, (2003)** found that increasing dietary lysine levels for broilers and balancing the essential amino acids improved FCR without modifying the feed intake because of the increase of muscular growth and body weight gain. Similarly, **El-Husseiny**

et al. (2004) and Yamazaki et al. (2006) found that feed intake of broiler chicks was not significantly affected by LCP amino acid supplemented diets. Also, Corzo et al. (2004, 2006) reported that increasing lysine only improved FCR without affecting FI. In addition, Sterling et al. (2006) found that increasing dietary lysine improved both FI and FCR and increased BWG. On the other hand, Khan et al. (2010) indicated that there was an increase in FI as CP levels were decreased from 23% to 19% but without affecting FCR.

Data of the dressed carcass of broiler chicks fed LCP-diets (10%) and supplemented with lysine (0, 0.2 or 0.4%) revealed that decreasing dietary protein significantly decreased carcass weight and increased abdominal fat. Lysine supplementation by 0.4% over that of NRC (1994) significantly improved dressed carcass and reduced abdominal fat (Table 3). Kerr and Kidd (1999) found that a reduction in dietary CP decreased carcass yields, increased abdominal fat, and decreased breast meat yield. Increasing fat accretion in broilers fed the LCP-diet may be attributed to increased feed intake as an attempt to meet their protein and amino acid requirement and the extra energy consumed will be deposited as fat. Similarly, it was found that abdominal fat was significantly increased in association with feeding low CP diet (18 to 16%) in the finisher period (Yonemochi et al., 2003; and Yamazaki et al., 2006). However, it is clear from our results that the LCP-diet supplemented with 0.4% lysine decreased carcass fat deposition near to that of the control diet.

#### Serum metabolites and HI titer

The study of the data related to serum metabolites (Table 4) showed that decreasing DCP level by 10% than that of NRC (1994) significantly ( $p < 0.05$ ) decreased serum total proteins, albumin and globulin and increased total lipids, triglycerides, cholesterol, HDL and LDL. Lysine supplementation by 0.4% above that of NRC (1994) to the LCP-diets increased serum total proteins. The increase in plasma proteins was concurrent with an increase ( $P < 0.05$ ) in plasma albumin (non specific immune factor) and an increase ( $P < 0.05$ ) in globulin, the specific immune factor and a decrease ( $P < 0.05$ ) in A/G ratio. The decrease in total lipids, triglycerides, cholesterol, HDL and LDL in broilers were not significantly ( $p < 0.05$ ) different from that of the broilers fed the normal recommended dietary protein and lysine levels. Thomas and Combs (1967) indicated that feeding a LCP-diet decreased total plasma protein and vice versa. This may be attributed to the finding of Harper (1975) who found that dietary protein served as a precursor for plasma proteins. However, Corzo et al. (2005) reported that total plasma protein was not affected by feeding LCP-diet supplemented with EAAs and NEAAs. On the other hand, Kaamal and Awadalla (2007) fed the broiler chicks 2% LCP diet with increasing utilizable lysine and methionine levels by 0.25 - 0.1% and 0.2 - 0.1% in starter and finisher diets respectively and found significant increases in serum total protein and albumin.

The effects of feeding the LCP-diet with lysine supplementation on haemagglutination inhibition (HI) titer to NDV are presented in table 4. At 7 day post vaccination (14 days of age), the HI titer was low for broiler chick fed

LCP-diet supplemented with 0 or 0.2% lysine. However, lysine supplementation by 0.4% of the diet increased HI titer. By 21 day of age, the HI titer of all treatment start to increase however the highest titer was for the control group followed by broiler chicks group fed LCP-diet supplemented with 0.4% lysine which were not significantly different from the control group. At 28 day of age, the HI titer of different broiler groups reach its highest value and the higher titer was for the broiler chicks fed the recommended CP and lysine levels followed by the broiler chicks fed the LCP-diet supplemented with 0.4% lysine and the lower HI titer was recorded for broiler chick fed the LCP diet supplemented with 0, or 0.2% lysine. **Carlomagno et al. (1980)** found that protein deficiency inhibited antibody production and the development of antibody producing cells in response to T-dependent antigens. **Aalbo and Kabasa (1996)** revealed that feeding broiler chicks vaccinated against Newcastle disease diets containing high ME/CP ratios

reduced IgM, IgG and HI titers. A deficiency of lysine may reduce immune response of the chicken and resulted in a reduced effect of vaccination. **Chen et al. (2003)** showed that lysine had an influence on chicken immune function and humoral immunity assay and cell mediated immunity function and showed a reduction in antibody response to NDV vaccination in broiler chickens fed a lysine deficient diet. Also, **Klaasing et al. (2000)** found that increasing the dietary concentration of lysine improved the haemagglutination and agglutinin titres, and IgG and IgM levels. Similarly, **Manzoor et al. (2003)** revealed that birds on high protein diets showed higher antibody and cell mediated responses than birds on low protein diets. From the present study it could be concluded that dietary protein level could be reduced by 10% of recommended level with supplementation of lysine at a level of 0.4 % above the recommended level with no adverse effect on growth performance, serum metabolites and immune response.

Table 1. Ingredient and chemical composition of the experimental diets provided with or without lysine for broiler chicks

Ingredients	Experimental diets											
	Starter				Grower				Finisher			
	Control	LP10% NL	LP10% HL0.2	LP10% HL0.4	Control	LP10% NL	LP10% HL0.2	LP10% HL0.4	Control	LP10% NL	LP10% HL0.2	LP10% HL0.4
Corn, yellow	54.00	59.21	59.00	58.21	61.30	63.4	63.20	63.00	65.50	69.57	69.02	69.00
Soybean meal	29.98	24.58	24.58	24.58	27.65	24.70	24.70	24.70	25.90	22.20	22.45	22.40
Fish meal	7.00	7.00	7.00	7.00	3.50	2.60	2.60	2.60	1.25	0.75	0.75	0.75
Soybean oil	5.10	5.63	5.62	6.02	5.20	5.55	5.55	5.7	4.75	4.50	4.50	4.50
Lime stone	1.87	2.30	2.31	2.25	1.90	2.00	2.00	1.95	2.00	2.18	2.18	2.00
Dica. phosphate	0.40	0.48	0.39	0.41	-	-	-	-	-	-	-	-
Common salts	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Min & Vit. premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L lysine HCl	-	0.15	0.45	0.75	-	0.1	0.35	0.60	-	0.15	0.45	0.70
DL methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
<b>Chemical composition (%)</b>												
CP	22.84	20.80	20.86	20.80	19.83	18.20	18.11	18.09	17.88	16.20	16.28	16.20
ME (Kcal/kg)	3198	3205	3202	3203	3197.79	3201.7	3191	3196	3192	3209.58	3196.73	3194.94
Ca	1.03	1.17	1.17	1.17	0.82	0.86	0.86	0.84	0.83	0.88	0.879	0.815
Available P	0.54	0.54	0.52	0.52	0.41	0.37	0.38	0.37	0.37	0.35	0.35	0.35
Lysine	1.30	1.30	1.50	1.70	1.09	1.94	1.22	1.40	0.93	0.92	1.148	1.326
Methionine	0.52	0.52	0.52	0.52	0.40	0.41	0.42	0.42	0.36	0.375	0.37	0.378

\*Mineral and vitamins premix each 2.5 kg/ton contained: choline, 300gm; Fe, 80gm; Mn, 60gm; Zn, 50gm; Cu, 8gm; I, 400mg; Se, 170mg; Co, 100mg; vitamin A, 12,000,000IU; vitamin D3 2,000,000 IU; vitamin E, 10 gm; vitamin K, 1g; vitamin B1, 2g; vitamin B2, 5g; vitamin B6, 1.5g; vitamin B12, 1g; BIOTIN, 5g; niacin, 30g; folic acid, 1gm; pantothenic acid 10gm.

Table 2. Effect of dietary protein level and lysine supplementation on the weekly body weight development, body weight gain (g) and feed conversion ratio of broiler chickens

Lysine supplementation % Exp. period	Dietary Protein & lysine levels (%)				
	Control	10% lower than recommended			
		0	0.2	0.4	
	Initial body weight, g	44.6 ± 0.42	43.39 ± 0.46	44.35 ± 0.54	44.66 ± 0.41
0-1	Body weight, g	134.9 <sup>a</sup> ± 1.97	133.9 <sup>ab</sup> ± 2.25	124.6 <sup>c</sup> ± 1.97	125.3 <sup>bc</sup> ± 1.89
	Body gain, g	90.31 <sup>a</sup> ± 1.81	88.58 <sup>ab</sup> ± 1.91	80.23 <sup>d</sup> ± 1.63	83.98 <sup>cd</sup> ± 1.624
	Feed consumption, g	123.00	160.44	138.42	142.7
	Feed conversion ratio	1.38 <sup>a</sup> ± 0.03	1.83 <sup>b</sup> ± 0.039	1.74 <sup>b</sup> ± 0.032	1.71 <sup>b</sup> ± 0.029
1-2	Body weight, g	327.69 <sup>a</sup> ± 5.02	281.7 <sup>d</sup> ± 6.05	285.18 <sup>cd</sup> ± 7.14	299.17 <sup>bc</sup> ± 7.86
	Body gain, g	192.73 <sup>a</sup> ± 3.61	147.7 <sup>d</sup> ± 3.67	162.06 <sup>cd</sup> ± 5.66	172.55 <sup>bc</sup> ± 6.23
	Feed consumption, g	290.45	322.33	327.77	314.4
	Feed conversion ratio	1.52 <sup>c</sup> ± 0.03	2.22 <sup>d</sup> ± 0.06	2.09 <sup>d</sup> ± 0.07	1.88 <sup>b</sup> ± 0.07
2-3	Body weight, g	615.3 <sup>a</sup> ± 12.52	547.5 <sup>b</sup> ± 14.08	559.83 <sup>b</sup> ± 16.05	592.02 <sup>ab</sup> ± 15.73
	Body gain, g	287.3 ± 9.23	263.8 ± 10.78	273.18 ± 11.71	290.75 ± 9.26
	Feed consumption, g	475.25	613.46	576.66	589.32
	Feed conversion ratio	1.68 <sup>a</sup> ± 0.058	2.40 <sup>b</sup> ± 0.095	2.21 <sup>ab</sup> ± 0.09	2.08 <sup>b</sup> ± 0.067
3-4	Body weight, g	1012.5 <sup>a</sup> ± 25.48	925.9 <sup>b</sup> ± 22.06	948.87 <sup>b</sup> ± 21.22	984.69 <sup>ab</sup> ± 24.79
	Body gain, g	397.53 ± 16.17	378.4 ± 12.76	389.03 ± 14.48	392.66 ± 17.07
	Feed consumption, g	690.66	960.35	918.61	873.73
	Feed conversion ratio	1.84 <sup>a</sup> ± 0.1	2.53 <sup>b</sup> ± 0.1	2.36 <sup>b</sup> ± 0.08	2.35 <sup>b</sup> ± 0.10
4-5	Body weight, g	1447.7 <sup>a</sup> ± 26.63	1286.3 <sup>d</sup> ± 27.75	1360.93 <sup>c</sup> ± 29.20	1390.35 <sup>bc</sup> ± 31.77
	Body gain, g	415.2 <sup>a</sup> ± 21.39	360.44 <sup>d</sup> ± 12.80	412.06 <sup>cd</sup> ± 19.30	403.65 <sup>cd</sup> ± 15.64
	Feed consumption, g	920.33	982.08	1074.85	990.36
	Feed conversion ratio	2.26 <sup>b</sup> ± 0.11	2.72 <sup>d</sup> ± 0.10	2.60 <sup>c</sup> ± 0.15	2.54 <sup>cd</sup> ± 0.094
5-6	Body weight, g	1941.8 <sup>a</sup> ± 37.36	1710.33 <sup>b</sup> ± 35.35	1836.66 <sup>b</sup> ± 32.27	1874.4 <sup>ab</sup> ± 41.20
	Body gain, g	494.14 <sup>a</sup> ± 16.50	423.66 <sup>b</sup> ± 15.54	475.73 <sup>b</sup> ± 20.99	484.4 <sup>ab</sup> ± 16.58
	Feed consumption, g	1090.05	1195.45	1287.14	1217.98
	Feed conversion ratio	2.29 <sup>b</sup> ± 0.09	2.82 <sup>d</sup> ± 0.09	2.70 <sup>c</sup> ± 0.17	2.60 <sup>cd</sup> ± 0.10

<sup>a, b, c, d</sup> means in the each row followed by a different superscript are significantly differ (p < 0.05).



Table 3. Effect of dietary protein level and lysine supplementation on growth performance and carcass quality (mean  $\pm$ SE) of broiler chickens.

Lysine supplementation %	Dietary Protein & lysine levels (%)			
	Control	10% lower than recommended		
		0	0.2	0.4
Items				
Live body weight (g)	1941.84 <sup>a</sup> $\pm$ 37.36	1710.33 <sup>c</sup> $\pm$ 35.35	1836.66 <sup>b</sup> $\pm$ 32.27	1874.26 <sup>ab</sup> $\pm$ 41.20
Body weight gain (g)	1897.19 <sup>a</sup> $\pm$ 37.26	1664.60 <sup>c</sup> $\pm$ 35.05	1792.31 <sup>b</sup> $\pm$ 32.02	1829.93 <sup>ab</sup> $\pm$ 40.97
Feed intake (g)	3590.34	4234.18	4323.45	4128.49
Feed conversion ratio	1.89 <sup>a</sup> $\pm$ 0.03	2.54 <sup>b</sup> $\pm$ 0.05	2.42 <sup>b</sup> $\pm$ 0.04	2.28 <sup>a</sup> $\pm$ 0.05
Protein efficiency ratio	2.87	2.32	2.49	2.62
Cost of Kg BW	3.81	5.12	4.94	4.71
Dressed carcass %	73.40 <sup>a</sup> $\pm$ 2.75	63.91 <sup>b</sup> $\pm$ 4.45	67.54 <sup>ab</sup> $\pm$ 2.44	69.58 <sup>ab</sup> $\pm$ 1.65
Abdominal fat%	1.10 <sup>a</sup> $\pm$ 0.11	1.88 <sup>b</sup> $\pm$ 0.14	1.50 <sup>b</sup> $\pm$ 0.08	1.33 <sup>bc</sup> $\pm$ 0.06

<sup>a,b,c</sup> means in the each row followed by a different superscripts are significantly differ ( $p < 0.05$ )

Table 4. Effect of dietary protein levels and lysine supplementation on serum metabolites and HIT of broiler chickens.

Lysine supplm. %	Diet. Protein & lysine levels (%)			
	Control	10% lower than recommended		
		0	0.2	0.4
Items				
Total protein (mg/dl)	4.04 <sup>a</sup> $\pm$ 0.14	2.49 <sup>c</sup> $\pm$ 0.10	2.64 <sup>c</sup> $\pm$ 0.18	2.88 <sup>bc</sup> $\pm$ 0.11
Albumin (g/dl)	1.75 <sup>a</sup> $\pm$ 0.03	1.45 <sup>c</sup> $\pm$ 0.07	1.48 <sup>bc</sup> $\pm$ 0.02	1.62 <sup>b</sup> $\pm$ 0.06
Globulin (g/dl)	2.28 <sup>a</sup> $\pm$ 0.09	1.04 <sup>b</sup> $\pm$ 0.07	1.17 <sup>a</sup> $\pm$ 0.14	1.26 <sup>bc</sup> $\pm$ 0.08
A/G ratio	0.76 <sup>a</sup> $\pm$ 0.11	1.38 <sup>b</sup> $\pm$ 0.11	1.28 <sup>bc</sup> $\pm$ 0.13	1.29 <sup>bc</sup> $\pm$ 0.07
Glucose (mg/dl)	102.86 $\pm$ 2.66	111.09 $\pm$ 4.23	109.49 $\pm$ 4.98	106.66 $\pm$ 3.32
Total lipid (g/dl)	398.59 <sup>a</sup> $\pm$ 7.05	529.34 <sup>b</sup> $\pm$ 5.96	503.24 <sup>b</sup> $\pm$ 7.26	448.82 <sup>a</sup> $\pm$ 8.95
Total cholesterol (mg/dl)	140.05 <sup>a</sup> $\pm$ 5.25	197.59 <sup>b</sup> $\pm$ 3.69	181.47 <sup>bc</sup> $\pm$ 6.39	172.23 <sup>bc</sup> $\pm$ 6.54
Triglycerides (mg/dl)	53.23 <sup>a</sup> $\pm$ 1.32	90.81 <sup>b</sup> $\pm$ 3.95	84.08 <sup>bc</sup> $\pm$ 2.57	77.37 <sup>b</sup> $\pm$ 1.92
HDLP (mg/dl)	86.05 <sup>a</sup> $\pm$ 3.21	110.65 <sup>b</sup> $\pm$ 4.40	102.65 <sup>bc</sup> $\pm$ 5.60	98.37 <sup>bc</sup> $\pm$ 3.75
LDL (mg/dl)	43.54 <sup>a</sup> $\pm$ 2.88	67.16 <sup>b</sup> $\pm$ 4.80	62.28 <sup>b</sup> $\pm$ 2.25	58.3 <sup>b</sup> $\pm$ 3.10
HI titer (log2) at 14 days	6.24 <sup>a</sup> $\pm$ 0.22	4.03 <sup>c</sup> $\pm$ 0.20	4.28 <sup>bc</sup> $\pm$ 0.31	5.55 <sup>b</sup> $\pm$ 0.34
HI titer (log2) at 21 days	6.62 <sup>a</sup> $\pm$ 0.25	5.05 <sup>c</sup> $\pm$ 0.22	5.26 <sup>bc</sup> $\pm$ 0.38	6.20 <sup>b</sup> $\pm$ 0.40
HI titer (log2) at 28 days	9.92 <sup>a</sup> $\pm$ 0.30	7.01 <sup>c</sup> $\pm$ 0.28	7.69 <sup>bc</sup> $\pm$ 0.40	8.94 <sup>b</sup> $\pm$ 0.47

<sup>a,b,c</sup> means in the each row followed by a different superscripts are significantly differ ( $p < 0.05$ ).

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