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Effects of Dietary Crude Protein on the Growth Performance, Carcass Characteristics and Serum Biochemical indexes of Lueyang Black-boned Chickens from Seven to Twelve Weeks of Age

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Keywords

Crude protein; Growth performance; Indigenous fowl; Lueyang black-boned chicken.

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ABSTRACT

This study was undertaken to assess dietary crude protein (CP) concentration for optimum growth performance and carcass characteristics of Lueyang black-boned chicken. In total, six hundred 42-day-old Lueyang black-boned chicks were randomly assigned to five treatments, each with six replicate pens with ten males and ten females. The birds fed experimental diets with different levels of protein concentration of 120, 140, 160, 180 and 200 g kg^{-1} from seven to twelve weeks of age respectively. On day of 84, weight gain, feed intake, and feed:gain ratio were measured, and two chickens (one male and one female) close to the average weight of all birds in each treatment were selected from each pen and sacrificed to evaluate carcass traits and selected serum biochemical indexes. Dietary CP concentration did not have any significant influence on feed intake ($p>0.05$). The birds fed the diet with 180 or 160 g kg^{-1} CP concentration exhibited greater ($p<0.05$) growth rate, better feed conversion ratio, relative breast weight and albumin concentration in serum than that of those fed other dietary CP concentrations. According to the results of regression analysis, the CP requirements of Lueyang black-boned chicken from seven to twelve weeks of age for optimal weight gain and feed:gain ratio were 174 and 170 g kg^{-1}, respectively.

INTRODUCTION

Black-boned chicken, called “Wuguji” in Chinese, is a kind of unique in indigenous fowl breeds in China. They can be easily distinguished from other chickens. The meat products of the original black-boned chicken are well known in the East and for thousands of years have been credited with famous medicinal and health-promoting effects. So far, more than ten such indigenous breeds have been discovered and characterized in different ecological regions of China. The origin of Lueyang black-boned chickens is the Shaanxi province of China. This breed is characterized by black-colored comb, beak, tongue, legs, toes, and skin. To their higher income and focused on healthy foods, more and more people have accepted and enjoyed this bird’s meat in China. So, such preference of consumers has strongly promoted the production of Chinese black-boned chickens. Because it is an indigenous fowl breed, research on the Lueyang black-boned chicken is very limited. Our previous work mainly focused on the genetic characteristics of this breed, including reproductive performance, meat production, etc. (Liu *et al.*, 1990; Liu *et al.*, 1992; Liu & Niu, 1996; Liu *et al.*, 2001). However, the knowledge on the nutritional physiology and how nutrients regulate growing, development, and live performance in Lueyang black-boned chicken is still lacking.

Protein is one of the most important dietary macronutrients for animals, and as the key component of cells, plays an important role in the process of life. Growth rate and feed efficiency of broilers improves with the increase in dietary protein, and there is a wealth of information about the effects of CP on the performance and body composition of chickens (Jackson *et al.*, 1982; MacLeod, 1990; Buyse *et al.*, 1992; Collin *et al.*, 2003; Swennen *et al.*, 2005; Niu *et al.*, 2009; Min *et al.*, 2012). Next to exogenous factors such as environmental conditions, particularly nutrition, genetic factors strongly influence the performance and body composition of chickens (Malone *et al.*, 1979; Jackson *et al.*, 1982; Barbato *et al.*, 1992; Hulan *et al.*, 1980). Relationships between genotype and dietary protein levels were also reported (Leclercq, 1983; Marks & Pesti, 1984; Cahaner *et al.,* 1987 and Smith & Pesti, 1998). Therefore, previous research has mainly focused on modern broiler chickens, but these results may be not true for indigenous fowl breeds, such as the Lueyang black-bone chicken because of significant differences in their physiology and genetics.

In the present study, the effect of different concentrations of dietary CP on the growth performance, carcass traits and selected blood biochemical indexes were evaluated in Lueyang black-boned chickens from seven to twelve weeks of age in order to determine their optimal protein requirement.

MATERIALS AND METHODS

Experimental design and birds

This experiment was conducted according to the regulations for the administration of affairs concerning experimental animals approved by State Scientific and Technological Commission (1988). Six hundred 42-day-old birds were selected and randomly allotted to thirty pens with ten male and ten female birds each. Experimental diets were based on corn and soybean meal (Table 1), five dietary treatments were formulated to contain equal metabolizable energy (ME) levels (12.34 MJ kg^{-1}), but protein levels of 120, 140, 160, 180, and 200 g kg^{-1}.

During experimental period, water and feed were provided *ad libitum*. The temperature was kept at 18-21ºC. The lighting program was gradually reduced from 16-18h to natural illumination (half an hour per week) during experimental period. At 42 d of age, birds were weighed. All of birds were reared under the similar conditions.

Table 1 – Composition of experimental diets fed to Lueyang black-bone chickens (7 to 12 wk of age)

	1	2	3	4	5
Ingredient (%)					
Corn	80.77	76.97	73.16	69.03	64.46
Wheat bran	6.66	4.96	3.01	2.00	1.72
Corn gluten meal	1.00	2.80	5.00	7.31	10.60
Soybean meal	7.94	11.67	15.02	18.00	19.70
Dicalcium phosphate	1.60	1.59	1.59	1.57	1.56
Limestone	0.66	0.63	0.61	0.59	0.58
Salt	0.37	0.37	0.37	0.37	0.37
Vitamin-mineral premix[1]	1.00	1.00	1.00	1.00	1.00
L-Lysine	-	-	0.16	0.07	-
DL-Methionine	-	-	0.08	0.04	-
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition[2]					
Metabolizable energy, MJ/kg	12.34	12.34	12.34	12.34	12.34
Crude protein, g/kg	120	140	160	180	200
Calcium, %	0.82	0.82	0.82	0.82	0.82
Nonphytate phosphorus, %	0.42	0.42	0.42	0.42	0.42
Lysine, %	0.45	0.55	0.80	0.80	0.80
Methionine, %	0.21	0.25	0.36	0.36	0.36

[1] Supplied the following per kilogram of total diet: vitamin A (retinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K_3 (menadione sodium bisulfate), 6 mg; thiamin, 6 mg; riboflavin, 12.85 mg; pyridoxine hydrochloride, 6 mg; cobalamin, 5 mg; folic acid, 6 mg; calcium-D-pantothenate, 12 mg; nicotinic acid, 40 mg; biotin, 5 mg; Cu ($CuSO_4 \cdot 5H_2O$), 7.68mg; Fe ($FeSO_4 \cdot 7H_2O$), 74 mg; Zn (ZnO), 76mg; Mn ($MnSO_4 \cdot H_2O$), 80 mg; Se ($NaSeO_3$), 0.0685 mg; I (KI), 0.2 mg; Ethoxycarbonyl quinoline, 2.8 mg; Limestone, 4.5g.

[2] The values are calculated according to feed composition and nutritive values in China (Ministry of Agriculture of China, 2011).

Measurements and sampling

Birds were weighed by pen at the beginning (42 d) and end (84 d) of the experiment after fasting for 12h (with free access to water). Feed intake per pen was recorded weekly. Body weight gain was measured at 84 d of age. Feed:gain ratio per pen was calculated for the overall experimental period.

After feed deprivation for 12h at 84 d of age, two chickens (one male and one female) close to the average weight of all birds in each treatment were selected from each pen, slaughtered, and the right *pectoralis major* muscle of the breast, right thigh (without bone) and abdominal fat pad were excised and weighed. In addition, 10mL blood samples were collected from a wing vein using a syringe. Blood was centrifuged after water bath for 2h at 37 ºC, and the serum was frozen (-20 ºC) until the analysis for biochemical indicators and hormone concentrations.

Serum biochemical indicators were determined with an automatic biochemical analyzer (HATICHI 7180, Japan) by using commercial kits: Total protein (TP) and albumin (ALB) concentrations were measured using TP kit and Alb kit (Biosino Bio-technology and Science INC, China). Blood urea nitrogen (BUN) and uric acid (UA) concentrations were determined using Urea kit and Uric acid kit (Shanghai Kehua Bio-engineering CO., LTD., China). Serum concentrations of triiodothyronine (T_3), thyroxine (T_4), and insulin (INS) were measured using an RIA kit (Beijing North Immunological Institute, China) and a gamma-counter (Packard 8500, USA).

Statistical analysis

All data were analyzed by one-way analysis of variance (SAS Version 9.1.3, 2004). When dietary treatment was significant ($p<0.05$), the test of *Duncan* for multiple comparisons was used to test the significance of the differences between treatment means (SAS Version 9.1.3, 2004). Linear regression analysis was applied to estimate the protein requirement of growing Lueyang black-bone chicken using the NLIN procedure of SAS software, according to the following quadratic model:

$$y = ax^2 + bx + c$$

where y=growth rate or feed:gain ratio; a and b are the coefficients of the quadratic equation; c is intercept; x is dietary protein level (g/kg) and -b/2a is x value for optimal response.

RESULTS

Growth performance and carcass traits

Body weight results obtained at 42 d and 84 of age are shown in Table 2. There was no significant difference in initial body weight (42 d) among treatments ($p>0.05$). Data on feed intake, weight gain, feed:gain ratio, and relative carcass part weights collected at 84 d of age are summarized in Table 3 and Table 4. It was clear that dietary CP did not have any significant influence on feed intake ($p>0.05$). The body weight of chickens fed the diet with 180 g kg^{-1} CP was significantly higher ($p<0.05$) than those of chickens fed the 120, 140, and 200 g kg^{-1} CP diets, but was not different from those fed the 160 g kg^{-1} CP diet.

Table 2 – Body weight of Lueyang black-boned chickens at 42 and 84 d of age

Dietary CP (g/kg)	Body weight 42d (kg/pen)	Body weight 84d (kg/pen)
120	7.10	19.46^{d}
140	7.14	21.43^{bc}
160	7.08	21.85^{ab}
180	7.10	22.66^{a}
200	7.12	20.89^{c}
SEM	0.01	0.57

$^{a-d}$ Means with different superscripts within the same column differ significantly ($p<0.05$).

Table 3 – Effect of dietary CP on weight gain, feed intake, and feed:gain of Lueyang black-boned chickens from 42 to 84 d of age

Dietary CP (g/kg)	Daily weight gain (g/bird per day)	Daily feed intake (g/bird per day)	Feed:gain (g:g)
120	15.24^{c}	65.82	4.32^{a}
140	17.31^{b}	67.90	3.92^{b}
160	17.58^{b}	65.52	3.73^{c}
180	18.84^{a}	69.38	3.68^{c}
200	17.40^{b}	68.38	3.94^{b}
SEM	0.48	10.76	0.02

$^{a-c}$ Means with different superscripts within the same column differ significantly ($p<0.05$).

The lower body weights of the low CP-fed (120 and 140 g kg^{-1} CP) chickens were also associated with significantly lower ($p<0.05$) relative breast muscle weight; however, relative breast muscle weights were not different between chickens fed the diets with 160 g kg^{-1}, 180 g kg^{-1}, and 200 g kg^{-1} CP. Dietary CP level did not influence thigh and abdominal fat pad weights ($p>0.05$).

Table 4 – Effect of dietary CP on breast meat, thigh meat, and abdominal fat pad of 84-d-old Lueyang black-boned chickens

Dietary CP (g/kg)	Relative weight (% of processing body weight)		
	Breast meat	Thigh meat	Abdominal fat pad
120	9.93^{c}	14.53	2.53
140	10.16^{bc}	15.61	2.30
160	10.98^{ab}	14.99	2.24
180	10.60^{ab}	13.61	2.47
200	11.50^{a}	15.76	1.14
SEM	0.92	2.99	1.84

$^{a-c}$ Means with different superscripts within the same column differ significantly ($p<0.05$).

The effect of CP level on the growth rate was significant ($p<0.05$). As with the body weight, the growth rate was significantly higher in the birds fed the diet with 180 g kg^{-1} CP compared with the other four diets. Over the entire experimental period, the effect of dietary protein level on feed:gain ratio was significant ($p<0.05$). The efficiency of feed utilization was higher (p<0.05) in chickens fed the diet having 180 g kg^{-1} CP, but there was no difference between this group and those fed with 20 g kg^{-1} less protein.

Moreover, regression analysis showed a highly significant (p<0.0001) correlation (R^2 = 0.6930) between growth rate (*y*) and protein level (*x*), as described by the following equation:

$$y = -0.00108x^2 + 0.37494x - 14.19616$$

The regression analysis of feed:gain ratio against protein level showed a highly significant relationship, and the following prediction equation:

$$y = 0.00025989x^2 - 0.08821x + 11.16961$$

(p<0.0001; R^2 =0.7075).

Serum biochemical parameters

Table 5 presents data on the obtained serum concentration of TP, ALB, globulin (GLO), BUN, UA, INS, T_3 and T_4. The concentrations of TP, GLO, BUN and UA were not affected by the treatments. However, TP and UA levels tended to reduce (though not significantly) when chickens were fed low CP diets. Conversely, increasing dietary CP level from 120 g kg^{-1} to 180 g kg^{-1} significantly (p<0.05) enhanced ALB concentration from 15.13 g L^{-1} to 17.72 g L^{-1}, but decreased to 17.38 g L^{-1} when dietary CP level was 200 g kg^{-1}. The concentration of T_3 was significantly (p<0.05) affected by dietary CP level. The concentrations of INS and T_4 were not affected by the treatments.

DISCUSSION

Several studies with poultry have investigated the effect of different dietary protein concentration in the diet on feed intake. The results of the present study suggest that feed intake may not be affected by increased dietary protein levels, and it corroborates with the findings of Rosebrough & Steele (1985), Fancher & Jensen (1989) & Bregendahl *et al.* (2002), who reported that chickens fed iso-energy diets with different protein concentrations had a similar feed intake. However, Smith & Pesti (1998) showed that feed intake of 18- to 53-day-old broiler chickens increased when dietary protein concentration decreased from 24 to 16% (at a constant energy level). Similar results were also observed by Parsons & Baker (1982), Pesti & Fletcher (1984), Suthama *et al.* (1991), Noy & Sklan (2002), Sklan & Plavnik (2002) and Swennen *et al.* (2004).

The growth rate of the chickens increased as the level of protein increased from 120 g kg^{-1} to 180 g kg^{-1} of the diet. This is consistent with the results reported by many other investigators (Yeh and Leveille, 1969; Jackson *et al.*, 1982; Buyse *et al.*, 1992; Smith and Pesti, 1998; Collin *et al.*, 2003; Swennen *et al.*, 2004). It is most likely the consequence of their increased cumulative protein consumption (Suthama *et al.*, 1991; Malheiros *et al.*, 2003).

During the overall experimental period, a linear reduction in feed:gain ratio was observed as growth rate increased when the level of protein increased from 120 g kg^{-1} to 180 g kg^{-1} of the diet. However, growth rate decreased when dietary protein level was increased to 200 g kg^{-1}, consequently increasing feed:gain ratio. This is likely due to the fact that the efficiency of dietary protein utilization decreased with each increment in dietary protein (Summers *et al.*, 1964; Jackson *et al.*, 1982; Swennen *et al.*, 2004).

An isoenergetic diet with varying CP levels, increasing dietary protein had no effect on relative abdominal fat pad weight. This supports earlier research carried out by Nawaz *et al.* (2006). However, other studies showed that broilers fed a low protein diet significantly increased abdominal fat pad weight compared with chickens fed a normal protein diet (Collin *et al.*, 2003; Swennen *et al.*, 2004, 2006). These differences may

Table 5 – Effect of dietary CP on biochemical indicators and hormone concentrations in the serum of 84-d-old Lueyang black-boned chickens[1]

Dietary CP (g/kg)	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	Urea nitrogen (mmol/L)	Uric acid (mmol/L)	Insulin (μIU/ml)	T_3 (ng/ml)	T_4 (μg/dl)
120	40.00	15.13[b]	24.87	0.45	0.14	8.30	1.56[ab]	7.75
140	39.23	15.88[b]	23.35	0.56	0.17	8.46	1.76[a]	7.41
160	43.53	17.30[a]	26.23	0.54	0.13	8.91	1.46[ab]	7.56
180	44.18	17.72[a]	26.46	0.49	0.18	6.41	1.64[a]	8.08
200	43.83	17.38[a]	26.45	0.54	0.22	8.89	1.31[b]	7.59
SEM	12.97	1.35	13.89	0.04	0.00	3.85	0.06	0.76

[a, b] Means with different superscripts within the same column differ significantly (p<0.05).

[1] T3= triiodothyronine; T4= thyroxine.

be due to by genotype or genotype × diet interactions (Leenstra, 1989; Marks, 1990).

Serum UA and TP concentrations tended to decrease (though not significantly) when chickens were fed the low protein diet. The level of ALB in the serum significantly decreased as dietary protein concentration was reduced. These results might be related with a deficit of amino acids intake by the animals (Corzo *et al.*, 2009). In other cases, broiler chickens reared on a low CP diet had significantly higher T_3 concentration and lower T_4 concentration compared with chickens fed a high CP diet (Keagy *et al.*, 1987; Rosebrough *et al.*, 1999; Malheiros *et al.*, 2003; Swennen *et al.*, 2005, 2006). In the present study, T_3 concentration was significantly affected by dietary CP level; however, T_3 concentration did not linearly increase with the increase in dietary CP level. Serum T_4 concentration was not affected by the treatments.

In conclusion, a diet with 180 or 160 g kg^{-1} CP concentration may improve growth rate and feed conversion ratio of growing Lueyang black-bone chickens, as well as increase their relative breast weight and serum albumin concentration. The regression analysis showed the CP requirements of Lueyang black-bone chickens from 42 to 84 of age for optimal weight gain and feed:gain ratio were 174 and 170 g kg^{-1}, respectively, when dietary metabolizable energy was 12.34 MJ kg^{-1}.

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