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Effect of Dietary Crude Protein Fluctuation on Performance, Blood Parameters and Nutrients Retention in Broiler Chicken During Starter Period

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Abstract: An experiment was conducted to evaluate the effect of three levels of CP (17.70, 20.80, 23.97) with constant essential amino acids on performance, blood parameters and nutrients retention of broiler chickens during starter period at 0-21 days of age. A completely randomized design with 3 dietary treatments and four replicates per treatment (10 birds per pen) was performed. Corn-soybean meal based diets fed to broiler chickens to provide 2900 kcal/kg ME. Chromium oxide was used at 18 days of age for measuring nutrient retention. Feed and water were provided *ad libitum* throughout the experiment. The average body weight gain (BWG), feed intake and water intake were recorded weekly. Blood samples were obtained at 21 days of age in heparinised tubes for measuring blood constituents. At this day, chickens from each replicate of treatment were randomly selected and tibia bone samples were taken for mineral retention study. Results of this experiment showed that CP content had no significant effect on feed intake (weekly and overall periods). Similar trend was observed for water intake except for 1-21 days, which 17.70% CP significantly decreased water intake ($P < 0.05$). Body weight gain (BWG) significantly reduced and FCR increased ($P < 0.05$) with decreasing CP. A change in CP content of the diets had no significant effect on blood parameters except for sodium (Na^+), potassium (K^+) and glucose (Glu) concentration. Increasing CP content of the diet significantly decreased P, Ca and N retention ($P < 0.001$). Increasing the CP content of the diet led to a decrease in length of tibia bone. Reducing the CP content of the diet, significantly decreased tibia bone P.

Key words: Crude protein fluctuation · Performance · Nutrient retention · Blood parameters · Broilers

INTRODUCTION

Environmental issues due to the emission of N, Ca and Av. P originated largely as a result of excess dietary crude protein (CP) and minerals from intensive livestock housing systems. The results of previous works [1-5] suggesting that dietary manipulation including reducing dietary CP of diets in poultry could be a useful tool to reduce NH_3 and consequently, other gas emissions and thus reduce concentration levels of aerial contaminants in and around poultry production systems. Jacob *et al*. [3] reported that the amount of N emitted in poultry manure can be reduced by up to 21% providing that CP content of the diet is lowered by 2.5%. However, the result of several experiments using low CP diet with broiler chickens [6, 7] have shown that growth performance and carcass composition of chickens become poorer when the dietary CP content is lowered by more than three to four percentage points. Therefore, it is generally not advisable to lower the dietary CP content by more than about three percentage points.

Reduction of phosphorus and Ca in broiler excreta is also desirable since excess levels may have a negative impact on the environment through leakage into waterways [8]. Reducing dietary calcium content may improve P retention [9, 10] and therefore, reducing P excretion. However, skeletal development in the growing bird may be affected by changes in mineral balance

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resulting from dietary manipulations to reduce pollution potential. On the other hands, the results of several experiments have shown that increasing CP [11] content by more than NRC [12] requirements may increase the performance and carcass composition of broilers.

Data regarding the interactive effects of CP by minerals on performance of broilers and retention of these elements in the gut of broilers is limited; therefore, the purpose of this experiment was to study the effect of different dietary CP content alone or in combination with different Ca and Av. P content (constant Ca to Av. P ratio) on growth and carcass characteristics, N, Ca and P retention and blood parameters of broiler chickens during the starter period.

MATERIALS AND METHODS

Dietary Treatments, Birds and Housing: In a completely randomised design (CRD) of three dietary treatments, this study was conducted over a 3-week period, using a total of 120 one-day-old male chickens of a commercial genotype (Ross 308). This experiment was conducted to examine the effect of three levels of crude protein (NRC, 15% more than NRC and 15% less than NRC recommendations) on performance, blood parameter and nutrient retention of broiler chickens from 1- 21 days of age. Each treatment group had four replicates (10 birds per pen of 1×1 m), allocated to one of 3 dietary treatments. The composition of dietary treatments is shown in Table 1. The mash form diets fed to broiler chickens were corn-soybean meal based and were formulated to provide 2900 kcal/kg ME. 0.3% chromium oxide as an indigestible marker was added to the diets at 18 days of age and fed to chickens for 3 days. Faeces samples were taken 3 times a day for 3 days, pooled together and kept in -20°C for later analysis. Feed and water were provided *ad libitum* throughout the experiment.

The average body weight gain (BWG), feed intake and water intake were recorded and expressed as weekly basis. The feed conversion ratio (FCR) was also calculated weekly. Chickens from each replicate pen were randomly selected and wing vein blood samples were obtained at 21 days of age after 3 hours of starvation. Blood in heparinised tubes was used for measuring blood constituents using a Stat Profile pHOx Plus L machine (*novo biochemical*, Novo International GmbH, Adam-Opel-Str., 19 A D53322 Rodermark, F. R., Germany, info@novobiochemical.de). At this day, chickens from each replicate of treatments were randomly selected and tibia bone samples were taken for mineral retention study. Ambient temperature was 29°C on day 1, gradually decreased to 21°C and then maintained at this level until the end of the experiment. Continuous lighting was used throughout the experiment. Apparent retention coefficients of N, Ca and P were estimated by using the formula of Sebastian *et al.* [13]. Tibia bones were removed

Table 1: Ingredients (%) and calculated analysis of the experimental diets for Ross male broiler chickens during starter period (0-21 days)

	Treatments		
Ingredients	20.80 CP[2] (%)	23.97 CP (%)	17.70 CP (%)
Corn	59.98	53.64	66.91
Soybean meal (44%)	28.09	30.37	24.88
Corn gluten	7.33	11.84	3.400
Wheat bran	0.00	0.00	0.00
Soya oil	1.00	0.80	0.95
Bone powder	1.97	1.91	2.03
Calcium carbonate	0.49	0.52	0.46
Vit. & min. premix[1]	0.50	0.50	0.50
Salt	0.42	0.42	0.42
DL Methionine	0.13	0.00	0.25
Lysine HCl	0.09	0.00	0.20
Calculated Analysis			
ME (Kcal/kg)	2900	2900	2900
CP (%)	20.8	23.97	17.7
Ca (%)	0.91	0.91	0.91
Av. P (%)	0.41	0.41	0.41
Na (%)	0.18	0.18	0.18

[1]Provided per kg of diet: vitamin A, 3,600,000 IU; vitamin D_3, 800,000 IU; vitamin E, 7,200 IU; vitamin K_3, 800 mg; vitamin B_1, 720 mg; vitamin B_2, 2,640 mg; vitamin B_3, 4,000 mg; vitamin B_5, 12,000 mg; vitamin B_6, 1,200 mg; vitamin B_9, 400 mg; vitamin B_{12}, 6 mg; vitamin H_2, 40 mg; choline chloride, 200,000 mg; Mn, 40,000 mg; Fe, 20,000 mg; Zn, 40,000 mg; Cu, 4,000 mg; Se, 80 mg [2]CP, crude protein

from the slaughtered birds and physical measurements of length, bone Ca and P were performed following ashing. The left tibias of each chicken used for measuring the percentage of tibia ash [14]. Briefly, bones were autoclaved under 1.32 pa pressure for 15 to 20 minutes.After cooling of the bones, they cleaned for adhering tissues and dried at 100°C for 48h. The tibias were then ashed in a muffle furnace overnight at 550°C and weighed again. Phosphorus concentrations in bones, feed and excreta were determined colourimetrically by the molybdo-vanadate method and the concentrations of Ca were determined using atomic absorption spectrophotometer [15]. Chromium oxide content in feed and excreta was measured according to Fenton and Fenton [16]. Nitrogen content of the samples was then determined using the Kjeldahl procedure described by AOAC [17]. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran.

Data Analysis: Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of Minitab [18]. Before analysing, the univariate test was used to assess the normality of all data.

Body weight, body weight gain, feed and water intake and feed conversion ratio were analysed on a floor pen basis, whilst blood and bone parameters were analysed on an individual bird basis.

RESULTS AND DISCUSSION

Performance: The effects of dietary CP on growth performance, feed and water intake and FCR are presented in Table 2. Dietary CP content had no significant effect on feed or water intake on days 1-7, 7-14 and 14-21. However, water intake was reduced when dietary CP reduced for the overall period (days 1-21). Decreasing the CP content of the diet had no significant effect on feed intake. Our finding concurs with some works demonstrated that the reduction in the crude protein levels in the diet does not affect feed intake of broiler chickens [19]. Although decreasing CP content had no significant effect (P >0.05) on weekly water intake, birds fed diets containing low protein diet drank less water compared with those of birds received NRC level or high CP content. Composition of a diet is an important factor that affect water intake. Therefore, lower water intake can be attributed to lower feed intake and CP content of the diet. The results of this study are in agreement with those of others [20] who demonstrated that broilers fed 17% CP diets, drank significantly less water than those received diets containing high CP (26%). Alleman & Leclercq [21] reported that broilers fed low CP content (16%) diets, drank lower water intake, independent of raising ambient temperature (22 or 32°C). The results of this study are also in agreement with those of others [22] who reported that reducing CP from 23 to 20.5% in broilers significantly decreased their water.

Table 2: Effects of dietary crude protein (CP%) on performance of Ross male broiler chickens during starter period (0-21 days)

Treatments	20.80 CP (%)	23.97 CP (%)	17.70 CP (%)	±SEM	P-value
Variables					
Feed intake (g)					
Day 1-7	107.5	92.4	95.0	5.800	0.224
Day 7-14	368.2	371.5	356.2	17.650	0.819
Day14-21	401.0	358.8	323.3	27.160	0.204
Day 1-21	877.4	822.7	774.4	41.560	0.289
Body weight gain (g)					
Day1-7	61.5	57.8	57.6	4.380	0.784
Day 7-14	122.8[a]	94.5[b]	88.9b	5.310	0.008
Day14-21	214.6[a]	146.2[b]	163.6[b]	8.610	0.003
Day 1-21	398.9[a]	298.4[b]	310.1[b]	17.330	0.012
Feed conversion ratio (g/g)					
Day1-7	1.76	1.62	1.66	0.100	0.633
Day 7-14	3.02[a]	3.99[b]	4.04[b]	0.150	0.005
Day14-21	1.89[b]	2.48[a]	1.99[b]	0.135	0.045
Day 1-21	2.21[a]	2.79[b]	2.51[ab]	0.109	0.027
Water intake (ml)					
Day1-7	305.5	305.4	276.3	11.920	0.217
Day 7-14	718.0	698.1	691.3	47.200	0.918
Day14-21	1434.0	1358.0	1194.0	70.930	0.125
Day 1-21	2458.0[a]	2361.0[a]	2161.0[b]	66.220	0.049

[a,b]Means in each row with no common superscripts are significantly different ($P < 0.05$)

Table 3: Effects of dietary crude protein (CP%) on blood parameters of Ross male broiler chickens during starter period (0-21 days)

Treatments	20.80 CP (%)	23.97 CP (%)	17.70 CP (%)	±SEM	P-value
Variables[1]					
pCo_2 (mmole/L)	43.5	42.7	43.4	1.63	0.920
pO_2 (mmHg)	51.3	51.8	50.7	3.98	0.983
sO_2 (%)	68.6	69.2	55.4	5.91	0.254
Het (%)	26.0	29.8	28.0	1.02	0.105
Hb (g/dL)	8.6	9.7	9.2	0.37	0.206
Na^+ (mmole/L)	166.2[a]	156.2[b]	170.3[a]	2.58	0.010
K^+ (mmole/L)	5.79[a]	7.78[b]	5.49[a]	0.35	0.007
Ca_2^+ (mmole/L)	1.07	1.06	1.06	0.17	0.999
Glu (mg/dL)	262.8[a]	290.5[b]	256.2[a]	6.89	0.027
Lac (mmole/L)	8.03	7.13	7.20	0.51	0.437
HCo_3^- (mmol/L)	22.5	22.6	24.3	0.93	0.367
pH	7.32	7.33	7.35	0.02	0.503

[1]pCo_2, blood carbon dioxide pressure; pO_2, blood oxygen pressure; sO_2, blood oxygen saturation;
Het, haematocrit; Hb, haemoglobin; Glu, blood glucose; Lac, blood lactose; HCo_3-, blood bicarbonate
[a,b]Means in each row with no common superscripts are significantly different ($P < 0.05$)

Dietary CP had a significant effect on body weight gain (BWG) and feed conversion ratio (FCR), except for the first 7 days. Birds fed diets containing 15% less CP than NRC and 15% more CP than NRC recommendations had lower BWG during days 7-14,14-21 and overall period of study (days 1-21) and higher FCR during days 7-14, 14-21. However, chickens fed diets containing 15% more CP than NRC recommendation had higher FCR in overall period of study (days 1-21) when compared with those of other treatments. These findings concur with those of others [6] who reported that reducing CP content of the diet by 2% decreased feed intake, BWG and increased FCR. However, Ferguson *et al.* [23] reported that increasing CP content of the diets from 22% to 26.4% had no significant effect on feed intake or BWG but the feed to gain ratio increased when CP of the diet decreased. Temim *et al.* [24] found that feeding broilers with high CP diets (25 *vs* 20%) at a high ambient temperature (32°C) during growing period improved weight gain. Temim *et al.* [25] reported an improvement in birds' performance when diets contained 28 and 33% CP, even when the birds were raised under heat stress.

Blood Parameters: The effects of treatments on blood parameters of broiler chickens at 21 days of age are presented in Table 3. Crude protein content of the diets had no significant effect on any blood parameters measured in the current experiment except for sodium, potassium and glucose concentrations. However, Birds fed 15% CP more than NRC recommendation had higher blood K^+ and glucose concentrations than those of other birds. These findings may corroborate the tendency of increasing blood components when their appropriate nutrients increase in the diet in modern broilers, which can improve the growth [26]. Increasing 15% CP content of the diets from NRC recommendation significantly (P <0.05) decreased blood Na^+ concentration as compare with those of control and 15% CP lower than NRC recommendation diets. It seems the homeostasis system of the chicken's body still can tolerate the CP changes and control the blood parameters measured in this study. These results, in support of previous findings [27] show that CP content of the diets can change the blood parameters of broiler chickens during the starter period.

Mineral Retention and Bone Parameters: The effects of CP content on mineral and nitrogen retention and tibia bone parameters on day 21 are presented in Table 4. The results show that dietary CP content had a significant effect ($P < 0.05$) on Ca and N retention. No significant effect on P retention was observed. In the current study, 15% increase in CP content of the diets significantly ($P < 0.05$) decreased Ca and N retention. Increasing CP content of the diet decreased N retention (50.5 in high CP content *vs* 63.3% in the control diet) and consequently, increased N excretion. These findings are in agreement with those of others who revealed high-protein diets may lead to an increase in nitrogen excretion [7] which may have a negative environmental impact.

In this study the length of the tibia was significantly ($P < 0.001$) decreased when CP content of the diets increased by 15%. This effect might be attributed to lower Ca retention in these birds. These results are in agreement with those of other studies which revealed high-protein diets may decrease the length of tibia bone [28]. The results of this study showed that CP content of the

Table 4: Effects of dietary crude protein (CP%) on nitrogen, Ca and P retention and tibia bone parameters of Ross male broiler chickens during starter period (0-21 days)

Treatments	20.80 CP (%)	23.97 CP (%)	17.70 CP (%)	±SEM	P-value
Variables					
Retention (%)					
P	51.8	45.9	53.9	2.84	0.199
Ca	49.9[a]	37.4[b]	57.5[a]	3.55	0.019
N	63.3[a]	50.0[b]	63.2[a]	2.73	0.021
Tibia bone parameters (day 21)					
Length (mm)	56.3[a]	52.7[b]	56.5[a]	0.63	0.009
Ash (%)	55.9	57.3	53.7	1.08	0.139
P (%)	16.08[ab]	16.69[a]	12.69[b]	0.93	0.046
Ca (%)	19.26	17.86	18.36	0.48	0.198

[a,b]Means in each row with no common superscripts are significantly different ($P < 0.05$)

diet had no significant effect on tibia ash. Similar trend was observed for tibia bone Ca. However, finding of this research was in contrast with Zyla *et al.* [29] who showed the overall mean values of toe ash were higher in birds at 21 days of age, consuming 0.59 or 0.69% dietary Ca than in those of birds fed 0.79% Ca and had higher Ca retention. However, decreasing CP content of the diet 15% lower that NRC recommendation led to 3.41% decrease in P content of the tibia as compare with the NRC CP recommendation.

CONCLUSIONS AND APPLICATIONS

Based on the data from this experiment, it was concluded that reducing CP content of the diets had no significant effect on feed and water intake. However, it reduced BWG and increased FCR. Moreover, reducing CP content of the diets had no significant effect on mineral and nitrogen retention. However, increasing CP content of the diet decreased BWG and increased FCR and resulted in a decrease in retention of Ca and N when compared with those of birds fed diets with lower CP content. Therefore, in poultry use of diets with lower CP could provide a method for lowering N and minerals excretion.

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