African Journal of Biotechnology Vol. 10(61), pp. 13342-13350, 10 October, 2011
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effects of dietary crude protein and calcium/phosphorus content on growth, nitrogen and mineral retention in broiler chickens

Nemat Ziaei[1]*, Hassan Kermanshahi[2] and Mohammad PileVar [2]

[1]Department of Animal Science, Jiroft Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.
[2]The Excellence Centre for Animal Sciences and Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, P.O. Box 91775-1163, Iran.

Accepted 11 August, 2011

This experiment was conducted to examine the effect of three levels of crude protein (CP) (NRC, 15% more than NRC and 15% less than NRC) and three levels of Ca and available P (Av. P) (NRC, 15% more than NRC and 15% less than NRC) on performance of broilers from hatching until 21 days of age. The experimental design was a completely randomized design (CRD), with a 3 × 3 factorial arrangement of nine dietary treatments. Each treatment combination had four replicate pens (10 birds per pen). Results of this experiment showed that CP content had no significant effect on feed and water intake. However, body weight gain (BWG) significantly reduced and FCR increased ($P < 0.05$) by 15% change in CP content of diet. Fifteen percent increase in mineral content of diets had no significant effect on feed intake. However, increase in Ca and Av. P significantly increased BWG and resulted in an improved feed conversion ratio (FCR) and increased water intake. There was an interactive effect of CP by Ca and Av. P levels on feed intake, BWG and FCR. A change in CP or mineral content of the diets had no significant effect on blood parameters except for potassium concentration (K^+). Reducing CP, Ca and Av. P content of the diet significantly ($P < 0.001$) increased P, Ca and N retention. Reduction in CP content of the diet led to a decrease in length, P and tibia bone ash. There was an interactive effect of CP by Ca and Av. P on the retention of Ca, N and ash of tibia bone.

Key words: Crude protein, Ca and Av. P levels, broilers, N retention.

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 (Reece et al., 1979; Gatel and Grosjean, 1992; Jacob et al., 1994; Smuts et al., 1995; Paul et al., 1996) 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. (1994) reported that the amount of N emitted in poultry manure can be reduced by up to 21% providing that CP content of diet is lowered by 2.5%. However, the result of several experiments using low CP diet with broiler chickens (Ferguson et al., 1998a; Aletor et al., 2000) 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 eutrophication of waterways (Catala´-Gregori et al., 2006). Reducing dietary calcium content may lead to improved P retention (Qian et al., 1997; Plumstead et al., 2008) and therefore, reduced P excretion. However, skeletal development in

*Corresponding author. E-mail: Nemat.Ziaei@ncl.ac.uk. Tel: +98-348-3260061. Fax: +98-348-3260065.

Table 1. Ingredients (%) and calculated analysis of the experimental diets for Ross male broiler chickens from 0 to 21 days of age.

Ingredient \ Treatment[1]	1	2	3	4	5	6	7	8	9
Corn	59.9	59.82	60.2	53.64	66.91	53.01	67.79	66.75	54.3
Soybean meal (44%)	28. 1	27.02	29.2	30.37	24.88	30.01	24.81	23.81	30.3
Corn gluten	7.33	8.12	6.54	11.84	3.40	12.19	3.33	4.19	11.6
Wheat bran	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15
Soya oil	1.00	1.00	1.00	0.80	0.95	1.00	0.64	0.95	0.56
Bone powder	1.97	2.42	1.51	1.91	2.03	2.36	1.59	2.48	1.47
Calcium carbonate	0.49	0.47	0.50	0.52	0.46	0.50	0.47	0.44	0.53
Vit and min. premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
DL Methionine	0.13	0.12	0.13	0.00	0.25	0.00	0.25	0.24	0.00
Lysine HCl	0.09	0.11	0.07	0.00	0.20	0.01	0.20	0.22	0.00
Calculated analysis									
ME (Kcal/kg)	2900	2900	2900	2900	2900	2900	2900	2900	2900
CP (%)	20.8	20.8	20.8	23.97	17.7	23.97	17.7	17.7	23.97
Ca (%)	0.91	1.05	0.77	0.91	0.91	1.05	0.77	1.05	0.77
Av. P (%)	0.41	0.47	0.35	0.41	0.41	0.47	0.35	0.47	0.35
Na (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Ca to Av. P ratio	2.22	2.22	2.22	2.22	2.22	2.22	2.22	2.22	2.22

[1]Treatments are: 1, NRC; 2, NRC with 15% more Ca and Av. P; 3, NRC with 15% less Ca and Av. P; 4, NRC with 15% more CP; 5, NRC with 15% less CP; 6, NRC with 15% more CP and 15% more Ca and Av. P; 7, NRC with 15% less CP and 15% less Ca and A. P; 8, NRC with 15% less CP and 15% more Ca and A. P; 9, NRC with 15% more CP and 15% less Ca and A. P. [2]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.

the growing bird may be affected by changes in mineral balance resulting from dietary manipulations to reduce pollution potential. On the other hands, the results of several experiments have shown that increasing CP (Holshemier and Veerkamp, 1992) or Ca and Av. P content by more than NRC (NRC, 1994) requirements may increase the performance and carcass composition of broilers.

As there are no data of the interactive effects of CP by minerals on the performance of broilers and retention of these elements in the gut of broilers; therefore, the purpose of this study 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 starting period.

MATERIALS AND METHODS

Dietary treatments, birds and housing

In a completely randomised design (CRD) experiment, with a 3 × 3 factorial arrangement of nine dietary treatments, this study was conducted over a 3-week period, using a total of 360 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) and three levels of calcium and available phosphorus (NRC, 15% more than NRC and 15% less than NRC with constant ratios) on performance of broiler chickens from hatching until 21 days of age. Each treatment group had four replicates (10 birds per pen of 1 × 1 m), allocated to one of 9 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. Chromium oxide of 0.3% 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 h of starvation. Blood in heparinized 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 treatment 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

Table 2. Effects of dietary crude protein (CP), calcium (Ca) and available phosphorus (Av. P) content on performance of Ross male broiler chickens from 0 to21 days of age.

Main effect	Feed intake (g/day)				Body weight gain (g/day)				Feed conversion ratio (day)				Water intake (ml/day)			
	1-7	7-14	14-21	1-21	1-7	7-14	14-21	1-21	1-7	7-14	14-21	1-21	1-7	7-14	14-21	1-21
CP (%)																
20.80	107.5	373.1	411.9	890.7	71.7	141.5	246.7^{a}	459.8^{a}	1.52	2.67^{a}	1.69^{b}	1.96^{a}	312.6	808.3	1443.0	2561.0
23.97	99.9	397.8	392.3	889.9	66.8	128.8	215.1^{b}	410.7^{b}	1.51	3.22^{b}	1.92^{a}	2.25^{b}	300.8	746.7	1412.0	2460.0
17.70	107.0	374.3	397.8	879.1	71.3	130.2	226.2^{b}	427.7^{b}	1.52	3.05^{b}	1.79^{b}	2.11^{c}	309.3	800.9	1343.0	2453.0
±SEM	2.87	9.92	10.08	18.87	1.93	4.88	5.21	9.74	0.044	0.089	0.054	0.046	9.19	25.49	34.82	53.06
P-values	0.134	0.141	0.380	0.889	0.159	0.154	0.001	0.005	0.981	0.001	0.023	0.001	0.650	0.215	0.133	0.290
Ca and Av. P (%)																
0.91 and 0.41	98.3^{a}	365.3	361.3^{a}	824.8^{a}	58.9^{a}	102.0	174.8^{a}	335.8^{a}	1.68^{a}	3.68^{a}	2.12^{a}	2.50^{a}	295.7	702.5^{a}	1329.0	2327.0^{a}
1.05 and 0.47	104.2^{ab}	388.2	426.2^{ab}	918.6^{ab}	75.1^{b}	151.0	258.6^{b}	484.6^{b}	1.39^{b}	2.59^{b}	1.65^{b}	1.90^{b}	302.0	820.2^{b}	1451.0	2574.0^{b}
0.77 and 0.35	111.9^{b}	389.9	414.5^{b}	916.3^{b}	75.7^{b}	147.5	254.6^{b}	477.9^{b}	1.48^{b}	2.66^{b}	1.63^{b}	1.92^{b}	325.0	830.3^{b}	1418.0	2573.0^{b}
±SEM	2.87	9.92	10.08	18.87	1.93	4.88	5.21	9.74	0.044	0.089	0.054	0.046	9.19	25.49	34.82	53.06
P-values	0.010	0.168	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.079	0.002	0.053	0.004

^{a,b,c}Means in each column not sharing a common superscript are significantly different (P < 0.05).

estimated by using the formula of this study (Sebastian et al., 1997). Tibia bones were removed from the slaughtered birds and physical measurements of length, bone Ca and P were measured following ashing. The left tibias of each chicken were used for measuring the percentage of tibia ash (Hall et al. 2003). Briefly, bones were autoclaved under 1.32 pa pressure for 15 to 20 min. After cooling of the bones, they were cleaned for adhering tissues and dried at 100°C for 48 h. 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 (AOAC, 1970). Chromium oxide content in feed and excreta was determined (Fenton and Fenton, 1979). Nitrogen content of the samples was then determined using the Kjeldahl procedure described (AOAC, 1995). 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 (v13, Minitab Inc., College, UK). Before analysing, the univariate test was used to assess the normality of all data (Minitab, 2004). 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

Performance

The main effects of dietary CP, Ca and Av. P on growth performance, feed and water intake and FCR are presented in Table 2. Dietary CP had no significant effect on feed or water intake on days 1 to 7, 7 to 14, 14 to 21 and the overall experiment (days 1 to 21). In the current experiment, reduction in CP content of the diet had no significant effect on feed intake. However, dietary CP had a significant effect on body weight gain (BWG) and feed conversion ratio (FCR). Birds fed diets containing 15% less CP than NRC recommendation had lower BWG during days 14 to 21 and the overall experiment (days 1 to 21), and higher FCR during days 7 to 14, 14 to 21 and the overall experiment (days 1-21).

Dietary Ca and Av. P levels (Table 2) had a significant effect (P < 0.05) on feed and water intake, BWG and FCR. Fifteen percent decrease

Table 3. Interactive effects of dietary crude protein (CP), calcium (Ca) and available phosphorus (Av. P) content on performance of Ross male broiler chickens from 0 to 21 days of age.

CP (%)	**20.8**	**20.8**	**20.8**	**23.97**	**17.7**	**23.97**	**17.7**	**17.7**	**23.97**		
Ca (%)	**0.91**	**1.05**	**0.77**	**0.91**	**0.91**	**1.05**	**0.77**	**1.05**	**0.77**		
Av. P (%)	**0.41**	**0.47**	**0.35**	**0.41**	**0.41**	**0.47**	**0.35**	**0.47**	**0.35**		
Variable[1]										**±SEM**	**P-value**
Feed intake (g)											
Days 1-7	107.5^{a}	a105.4	109.5^{a}	a92.4	107.4^{a}	99.8^{a}	a95.0	99.9^{a}	126.3^{b}	4.98	0.011
Days 7-14	368.2	363.1	382.8	371.5	424.2	397.6	356.2	377.3	389.4	17.18	0.496
Days 14-21	b401.7^{a}	a408.6	425.4^{a}	358.8$^{b\ c}$	429.1^{a}	388.9^{a}	323.3^{c}	440.8^{a}	429.3^{a}	17.46	0.028
Days 1-21	877.4	877.1	917.6	822.7	960.8	886.3	774.4	918	944.9	32.68	0.078
Body weight gain (g)											
Days 1-7	61.5	75.4	78.3	57.8	73.2	69.4	57.6	76.8	79.5	3.33	0.59
Day 7-14	122.7	146.1	155.5	94.5	153.1	139	88.9	153.7	148	8.45	0.142
Days 14-21	214.6^{b}	253.9^{a}	271.5^{a}	146.2^{c}	261.1^{a}	238.1^{ab}	163.6^{c}	260.0^{a}	254.4^{a}	9.03	0.005
Days 1-21	398.9^{b}	475.4^{a}	505.3^{a}	298.4^{c}	487.4^{a}	446.4^{a}	310.1^{c}	491.1^{a}	481.9^{a}	16.88	0.018
Feed conversion ratio (g/g)											
Days 1-7	1.75	1.4	1.4	1.62	1.47	1.44	1.66	1.31	1.6	0.077	0.169
Days 7-14	3.02^{c}	2.51^{a}	2.47^{a}	3.99^{b}	2.79^{a}	2.86^{a}	4.04^{b}	2.46^{a}	2.66^{a}	0.154	0.023
Days 14-21	1.89^{b}	1.61^{a}	1.57^{a}	2.48^{c}	1.64^{a}	1.63^{a}	1.99^{b}	1.69^{a}	1.69^{a}	0.093	0.015
Days 1-21	2.21	1.85	1.82	2.79	1.97	1.98	2.51	1.87	1.96	0.079	0.059
Water intake (ml)											
Days 1-7	305.5	313.6	318.8	305.4	291.3	305.9	276.3	301.3	350.5	15.91	0.2
Days 7-14	718	810.8	887.1	698.1	775.6	766.5	691.3	874.1	837.4	44.15	0.531
Days 14-21	1434	1431	1464	1358	1472	1408	1194	1451	1383	60.31	0.309
Days 1-21	2458	2556	2670	2361	2539	2480	2161	2626	2571	91.9	0.277

[1 a,b, c] Means in each row not sharing a common superscript are significantly different (P < 0.05).

in dietary Ca/Av. P content significantly increased feed intake (P < 0.05) during days 1 to 7 (P < 0.01), 14 to 21 (P < 0.001) and the overall experiment (P < 0.01) and consequently, BWG (P < 0.001) during days 1 to 7, 14 to 21 and the overall experiment (days 1 to 21). The interactive effect of dietary CP, Ca and Av. P content on performance of the broiler chickens is presented in Table 3. Dietary CP, Ca and Av. P content had a significant effect (P < 0.05) on feed intake, BWG and FCR but had no significant effect on water intake. Birds fed diets containing 23.97 CP, 0.77 Ca and 0.35% Av. P had higher feed intake during days 1 to 7 (P < 0.01), 14 to 21 (P < 0.05) and the overall experiment (P = 0.078) and consequently higher BWG during days 14-21 (P < 0.05) and overall the experiment (P < 0.05). These birds had an improved FCR during days 7 to 14 (P < 0.05), 14 to 21 (P < 0.05) and the overall experiment (P = 0.059) when compared with control diet (Table 1, T1; NRC recommendation).

Blood parameters

The results of main effects of CP, Ca and Av. P on blood parameters of broiler chickens at 21 days of age are presented in Table 4. CP or Ca and Av. P content of the diets had no significant effects on any blood parameters measured in the current experiment.

The interactive effect of CP, Ca and Av. P content of the diets on blood parameters of broiler chickens at 21 days of age are shown in Table 5. No interactive effect of CP by Ca and Av. P on any measured blood parameters except for K^+ concentration that was observed in this experiment. Birds fed 15% CP more than NRC and Ca and Av. P based on NRC recommendation had higher blood K^+ concentration than those of other birds.

Table 4. Effects of dietary crude protein (CP), calcium (Ca) and available phosphorus (Av. P) content on blood parameters of Ross male broiler chickens from 0 to 21 days of age.

CP (%)	**20.8**	**23.97**	**17.70**		
Variable[1]				**±SEM**	**P-value**
pCo_2 (mmole/L)	43.02	42.18	43.72	1.1	0.62
pO_2 (mmHg)	52.98	50.73	49.88	2.237	0.605
sO_2 (%)	62.48	63.88	63.60	5.222	0.98
Het (%)	26.17	27.5	27.67	1.037	0.542
Hb (g/dL)	8.64	9.02	9.17	0.336	0.532
Na^+ (mmole/L)	164.1	159.7	166.2	2.67	0.234
K^+ (mmole/L)	6.06^a	6.56^{ab}	6.69^b	0.199	0.018
Ca^{2+} (mmole/L)	1.29	1.61	1.18	0.08	0.475
Glu (mg/dL)	270.4	278.9	261.4	4.937	0.061
Lac (mmole/L)	7.24	6.91	7.03	0.358	0.802
HCo_3^- (mmol/L)	24.28	22.88	24.39	0.668	0.222
pH	7.36	7.34	7.35	0.016	0.685
Ca (%)	**0.91**	**1.05**	**0.77**		
Av. P (%)	**0.41**	**0.47**	**0.35**		
Variable				**±SEM**	**P-value**
pCo_2 (mmole/L)	43.19	42.88	42.85	1.1	0.97
pO_2 (mmHg)	51.26	52.9	49.44	2.237	0.558
sO_2 (%)	64.42	66.56	58.98	5.222	0.579
Het (%)	27.92	27.33	26.08	1.037	0.454
Hb (g/dL)	9.17	9.03	8.63	0.336	0.504
Na^+ (mmole/L)	164.3	164.7	161.0	2.67	0.559
K^+ (mmole/L)	6.35	5.88	6.08	0.199	0.259
Ca^{2+}(mmole/L)	1.06	1.31	1.26	0.08	0.085
Glu (mg/dL)	269.8	272.7	268.3	4.94	0.816
Lac (mmole/L)	7.45	7.03	6.70	0.358	0.348
HCo_3^- (mmol/L)	23.16	24.03	24.36	0.668	0.434
pH	7.33	7.35	7.36	0.016	0.514

[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 not sharing a common superscript are significantly different (P < 0.05).

Mineral retention and bone parameters

The main effect of CP, Ca and Av. P content on mineral and nitrogen retention and tibia bone parameters on day 21 are presented in Table 6. The results show that dietary CP content had a significant effect ($P < 0.001$) on Ca, P and N retention. In the current experiment, 15% decrease in CP content of the diets significantly ($P < 0.05$) increased P, Ca and N retention. Increasing CP content of the diet resulted in a decrease in N retention (57.9 in high CP content vs. 64.3% in the control diet) and consequently, increasing N excretion. However, tibia ash significantly ($P < 0.05$) increased possibly due to 15% augmenting of CP content of the diet. Decreasing CP content of the diet led to 2.4% decrease in P content of the tibia. Reducing Ca and Av. P levels of the diets had no significant effect on P retention. However, reducing Ca and Av. P levels by 15% resulted in an increase in Ca ($P < 0.01$) (from 48.3 in the control diet to 64.2% in reduced Ca diet) and N ($P < 0.01$) (from 58.8 in the control diet to 64.1% in reduced Ca diet) retention.

The interactive effects of CP, Ca and Av. P contents on mineral and N retention and tibia bone parameters are presented in Table 7. There was no significant interactive effect of CP by Ca and Av. P on P retention. However, diet containing 23.97% CP, 0.77% Ca and 0.35% Av. P tended ($P = 0.083$) to have higher P retention when compared with control or other dietary treatments.

DISCUSSION

Performance

In the current experiment, our finding concurs with some works that demonstrated that reduction in the crude

Table 5. Interactive effects of dietary crude protein (CP), calcium (Ca) and available phosphorus (Av. P) content on blood parameters of Ross male broiler chickens from 0 to 21 days of age.

CP (%)	**20.8**	**20.8**	**20.8**	**23.97**	**17.7**	**23.97**	**17.7**	**17.7**	**23.97**		
Ca (%)	**0.91**	**1.05**	**0.77**	**0.91**	**0.91**	**1.05**	**0.77**	**1.05**	**0.77**		
Av. P (%)	**0.41**	**0.47**	**0.35**	**0.41**	**0.41**	**0.47**	**0.35**	**0.47**	**0.35**		
Variable[1]										**±SEM**	**P-value**
pCo_2 (mmole/L)	43.53	43.65	41.88	42.65	41.35	42.55	43.4	43.63	44.13	1.905	0.93
pO_2 (mmHg)	51.27	54.15	53.52	51.77	49.93	50.5	50.7	54.63	44.3	3.875	0.592
sO_2 (%)	68.6	73.38	45.48	69.23	67.03	55.38	55.4	59.28	76.1	9.044	0.104
Het (%)	26	26.75	25.75	29.75	27.5	25.25	28	27.75	27.25	1.796	0.778
Hb (g/dL)	8.63	8.85	8.45	9.7	9.08	8.28	9.18	9.18	9.15	0.582	0.764
Na^+ (mmole/L)	166.6	165.3	160.4	156.2	164.4	158.6	170	164.5	164	4.631	0.628
K^+ (mmole/L)	5.79[a]	6.14[a]	6.25[a]	7.78[b]	5.83[a]	6.06[a]	5.4[a]	5.67[a]	5.92[a]	0.346	0.008
Ca^{2+} (mmole/L)	1.07	1.43	1.38	1.06	1.26	1.17	1.06	1.26	1.24	0.139	0.946
Glu (mg/dL)	262.8	273.5	275	290.5	279.5	266.8	256	265	263	8.55	0.294
Lac (mmole/L)	8.03	6.88	6.83	7.13	7.15	6.45	7.2	7.05	6.83	0.621	0.876
HCo_3^- (mmol/L)	22.53	25.43	24.9	22.63	22.03	23.98	24.3	24.65	24.2	1.157	0.506
pH	7.32	7.37	7.38	7.33	7.32	7.36	7.35	7.35	7.34	0.028	0.671

[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 not sharing a common superscript are significantly different ($P < 0.05$).

Table 6. Effects of dietary crude protein (CP), calcium (Ca) and available phosphorus (Av. P) content on nitrogen, mineral retention and tibia bone parameters of Ross male broiler chickens at 21 days of age.

CP (%)	**20.8**	**23.97**	**17.7**		
Variable				**SEM±**	**P-value**
Retention (%)					
P	41.6[a]	48.8[b]	56.2[b]	3.04	0.009
Ca	58.8[a]	41.5[b]	63.3[a]	2.97	0.001
N	64.3[a]	57.9[b]	67.9[a]	1.31	0.001
Tibia bone parameters (day 21)					
Length (mm)	59.2	56.7	57.1	0.74	0.062
Ash (%)	54.2[a]	56.0[b]	53.6[a]	0.59	0.019
P (%)	16.4[a]	16.3[a]	14.0[b]	0.61	0.015
Ca (%)	19	18.4	18.3	0.36	0.366
Ca (%)	**0.91**	**1.05**	**0.77**		
Av. P (%)	**0.41**	**0.47**	**0.35**		
Variable				**SEM±**	**P-value**
Retention (%)					
P	50.5	46.8	49.3	3.04	0.681
Ca	48.3[a]	51.1[a]	64.2[b]	2.97	0.002
N	58.8[a]	67.2[b]	64.1[b]	1.31	0.001
Tibia bone parameters (day 21)					
Length (mm)	55.1[a]	58.6[b]	59.2[b]	0.75	0.001
Ash (%)	55.6	54	54.2	0.59	0.135
P (%)	15.2	15.7	15.8	0.61	0.723
Ca (%)	18.5	18.4	18.9	0.36	0.605

[a,b] Means in each row not sharing a common superscript are significantly different ($P < 0.05$).

Table 7. Interactive Effects of dietary crude protein (CP), calcium (Ca) and available phosphorus (Av. P) content on nitrogen, mineral retention and tibia bone parameters of Ross male broiler chickens at 21 days of age.

CP (%)	**20.8**	**20.8**	**20.8**	**23.97**	**17.7**	17.70	17.7	**23.97**		
Ca (%)	**0.91**	**1.05**	**0.77**	**0.91**	**0.91**	0.77	1.05	**0.77**		
Av. P (%)	**0.41**	**0.47**	**0.35**	**0.41**	**0.41**	0.35	0.47	**0.35**		
Variable									**±SEM**	**P-value**
Retention (%)										
P	51.8	42.3	40.6	45.9	54.7	53.9	53.3	61.5	5.27	0.083
Ca	49.9[ab]	66.9[b]	59.5[b]	37.4[a]	40.0[a]	57.5[a]	46.5[a]	86.0[c]	5.15	0.002
N	63.3[ac]	67.0[ad]	62.6[ac]	50.0b	66.0[ad]	63.2[a]	68.6[ad]	72.0[d]	2.27	0.023
Tibia bone parameters (day 21)										
Length (mm)	56.3	1.05	61	52.7	57.9	56.5	57.6	57.2	1.35	0.226
Ash (%)	55.9[bc]	0.47	53.8[ab]	57.3[bc]	57.1[bc]	53.7[ab]	52.1[a]	55.0[bc]	1.02	0.041
P (%)	16.1	16.6	16.5	16.7	16.1	12.7	14.3	14.9	1.06	0.729
Ca (%)	19.3	18.3	19.4	17.9	18.3	18.4	18.5	18.1	0.62	0.580

[a,b,c,d] Means in each row not sharing a common superscript are significantly different (P < 0.05).

protein levels in the diet does not affect feed intake of broiler chickens (Blair et al., 1999). Although, decreasing CP content had no significant effect (P > 0.05) on water intake, birds fed diets containing low protein diet had lower drinking water when compared with the groups that received NRC level or high CP content. An important factor that is affecting water intake is the nutritional composition of diet; 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 Marks and Pesti (1984) who demonstrated that broiler fed diets with 17% CP drank significantly less water than those received diets containing high CP (26%). Alleman and Leclercq (1997) reported that broiler 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 Ziaei et al. (2008), who reported that reducing CP from 23 to 20.5% significantly resulted in a decrease in water intake in broilers. In the experiment, the lower BWG and higher FCR for the birds that received the mentioned diets can be attributed to the lower feed intake. As shown in Table 2, 15% decrease in CP content of the diets resulted in a decrease in feed intake. These findings concur with those of Ferguson et al. (1998a) who reported that reducing CP content of diet by 2% decreased feed intake, BWG and increased FCR. However, Ferguson et al. (1998b) reported that increasing CP content from 22 to 26.4% had no significant effect on feed intake or BWG, but for the feed to gain ratio, it increased by reducing the CP of the diet. Temim et al. (1999) found that feeding broilers with high CP diet (25 vs. 20%) at a high ambient temperature (32°C) during growing period improved weight gain. Temim et al. (2000) reported an improvement in birds' performance when diets contained 28 and 33% of CP, even when the birds were raised under heat stress. Birds fed diets containing 15% Ca and Av. P less than NRC recommendation had an improved FCR (P < 0.001) and drank more water during days 7 to 14 (P < 0.01) and the overall experiment (P < 0.01). The higher BWG and water intake can be attributed to the higher feed intake or Ca and Av. P content. Ziaei et al. (2008) reported that reducing dietary Ca content from 0.90 to 0.73% and Av P from 0.45 to 0.335% significantly (P < 0.01) decreased water intake during the grower period, which may be attributable to the reduction in feed intake observed with reduced dietary mineral content. Reducing CP, Ca and Av. P of the diets resulted in a decrease in feed and water intake, BWG and increase in FCR. The results of this study concur with those of Ferguson et al. (1998a) who reported that reducing CP and P concentration of the broiler diets was associated with a decrease in body weight at 21 days of age. In the current experiment, increasing CP or Ca/Av. P content of the diet led to more water intake, although, it was not significant. These findings are similar to those of Ziaei et al. (2006) who reported that chicks fed diet containing 23.0 CP, 0.95 Ca and 0.45% Av. P during the starter period had higher water intake when compared with those received diets consisting of 20.5, 0.85 and 0.335% CP, Ca and Av. P, respectively. Higher water intake can be attributed to feed intake, CP or mineral content of the diets.

Blood parameters

Fifteen percent decreases in CP content of the diets significantly ($P < 0.05$) increased blood K^+ concentration. It seems the homeostasis system of the chicken body can still tolerate the CP, Ca and Av. P changes and control the blood parameters measured in this study. Our findings are similar to those of Ziaei et al. (2008) who reported that apart from plasma P level on day 21, dietary mineral content had no significant effect on any of the blood parameters measured. In the current experiment, change in Ca and Av P content of diet increased blood Ca concentration (1.06, 1.31 and 1.26 in control, 15% more than NRC and 15% less than NRC recommended diets, respectively) although, these changes were not significant ($P > 0.05$). The results of this study are in consistency with those of Ziaei et al. (2008) who reported that reducing dietary Ca and P content had no significant effect on most of the blood parameters recorded, apart from plasma P at the end of the grower period which was significantly lower in the reduced P diets. Shafey et al. (1990) reported that increasing the Ca level of diets from 1.2 to 2.2% significantly increased total plasma Ca concentration, but only dietary Ca at the level of 3.2% increased plasma iCa (ionized Ca) and reduced P concentration. Increasing Av. P content from 0.40 to 0.85% significantly reduced plasma total Ca and iCa and increased plasma P. Sebastian et al. (1996) reported a decrease in plasma P and a significant increase in plasma Ca of broiler chickens fed a low P diet (0.3% Av. P). A low P diet normally results in elevated plasma iCa which depresses the release of parathyroid hormone (PTH), thus, reducing the PTH inhibition on tubular reabsorption of phosphate and permitting the urinary excretion of additional Ca be absorbed from the gut (Sebastian et al., 1996).

Mineral retention and bone parameters

These findings are in agreement with the research which revealed that high-protein diets may lead to an increase in nitrogen excretion (Aletor et al., 2000) which has a negative environmental impact. In the current experiment, CP content of the diet had no significant effect on the length of the tibia on day 21. Reducing Ca and Av. P levels of the diets had no significant effect on P retention. However, reducing Ca and Av. P levels by 15% resulted in an increase in Ca ($P < 0.01$) (from 48.3 in the control diet to 64.2% in reduced Ca diet) and N ($P < 0.01$) (from 58.8 in the control diet to 64.1% in reduced Ca diet) retention. Ferguson et al. (1998a) reported that a reduction in dietary P resulted in a significant decrease in P excretion in the litter (23.2%). In the current experiment, the length of the tibia was significantly ($P < 0.001$) increased by 15% reduction in Ca and Av. P concentration of the diets and birds fed diet containing lower Ca and Av. P content had a longer tibia bone. This effect might be attributed to higher Ca retention in these birds. Reducing Ca and Av. P levels of diets had no significant effect on ash or Ca and P content of the tibia. Zyla et al. (2000) found that 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 fed 0.79% Ca.

There was no significant interactive effect of CP by Ca and Av. P on P retention. However, diet containing 23.97% CP, 0.77% Ca and 0.35% Av. P tended ($P = 0.083$) to have higher P retention when compared with control or other dietary treatments. Our finding is similar to that of Ferguson et al. (1998a) who reported that a reduction in dietary P resulted in a significant decrease in P excretion in the litter (23.2%). However, this response depended on the CP content of the diet, as there was a significant CP by P interaction. Dietary treatments had a significant effect ($P < 0.05$) on Ca and N retention. Our results concur with those of Mitchell and Edwards (1996) who reported that reduced mineral content of diets resulted in a higher apparent retention of Ca and P. Ziaei et al. (2008) reported that reducing dietary Ca and P content had no significant effect on metabolisability coefficients of N, Ca and P. However, P excretion was decreased by reducing P content of the diet. Ferguson et al. (1998a) reported that the N content of litter was significantly ($P < 0.001$) reduced by decreasing the CP content of diet and for each percentage point reduction in dietary CP (supplementing with AA) there will be 7% reduction in the N content of litter and similar to the N, a reduction in dietary P resulted in a significant ($P < 0.001$) in P excretion. In the current study, the highest Ca and N retention was related to those of birds fed dietary treatment 9 (15% more than NRC for CP and 15% less than NRC for Ca and Av. P). Dietary treatments had no significant effect on the length of P and Ca content of the tibia. Araujo et al. (2003) found no significant interaction of amino acids and Ca levels on the length and thickness of the compact and spongy layers of the tibia at day 21 in broilers. In this study, there was a significant effect ($P < 0.05$) for dietary treatments on tibia ash and those of birds fed Ca and Av. P based on NRC recommendations had higher tibia ash than those that received other dietary treatments. Reducing Ca and Av. P content of the diet significantly increased the length of the tibia which can be attributed to the higher retention of Ca or P in the gut of birds that received reduced Ca and Av. P diet. The results of this study are in agreement with those of Ziaei et al. (2008) who reported that reducing Ca and P levels had major effects on bone characteristics. At day 21, diameter, thickness, bone strength and flexural stiffness were significantly lower in broilers fed diets with lower Ca and P content. The different findings might be attributed to the methods used for the experiment, Ca and Av. P content of the diet and possibly the feedstuffs fed by chickens.

Based on the data from this experiment, it was concluded that reducing CP had no significant effect on feed and water intake. However, it reduced BWG and increased FCR. The reduced Ca and Av. P content of the diet under current standards led to increase in feed and water intake, BWG and improved FCR. Decreasing CP and Ca and Av. P resulted in a significant increase in N, Ca and P retention. However, increasing CP content of the diet decreased BWG and increased FCR, and also resulted in a decrease in retention of Ca, P and N when compared with reduced CP or minerals content. Therefore, the use of reduced CP and minerals diets in poultry could provide a method for lowering N and minerals excretion.

ACKNOWLEDGEMENTS

The authors would like to thank the Shahid Bahonar University of Kerman, Iran and the Ferdowsi University of Mashhad, Iran for financial and technical support of the project and also Dr. Samuel Arkle in the UK for reading the article.

REFERENCES

Aletor VA, Hamid II, Nieb E, Pfeffer E (2000). Low-protein amino acid supplemented diets in broiler chickens effects on performance, carcass characteristics, whole body composition and efficiencies of nutrient utilization. J. Sci. Food Agric. 80: 547-554.

Alleman F, Leclercq B (1997). Effect of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. Br. Poult. Sci. 38: 607-610.

AOAC (1995). Official Methods of Analysis. 8th ed. Association of Official Agricultural Chemists, Washington, DC, USA.

AOAC (1970). Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, VA.

Araujo M, Lousada JQ, Junqueira OM, Rodriguez AIR (2003). Bone development of broiler chickens fed diets with different amino acids and calcium levels during the starter phase. Int. J. Morphol. 21: 101-106.

Blair R, Jacob JP, Ibrahim S, Wang P (1999). A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. J. Appl. Poult. Res. 8: 25-47.

Catala-Gregori P, Garcia V, Hernandez F, Madrid J, Ceron JJ (2006). Response of broilers to feeding low-calcium and phosphorus diets plus phytase under different environmental conditions Body weight and tibiotarsus mineralization. Poult. Sci. 85: 1923-1931.

Fenton TW, Fenton M (1979). An improved procedure for the determination of chromic oxide in feed and excreta. Canadian. J. Anim. Sci. 59: 631-634.

Ferguson NS, Gates RS, Taraba JL, Cantor AH, Pescatore AJ, Straw ML, Ford MJ, Burnham DJ (1998a). The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers. Poult. Sci. 77: 1085-1093.

Ferguson NS, Gates RS, Taraba JL, Cantor AH, Pescatore AJ, Straw ML, Ford MJ, Burnham DJ (1998b). The effect of dietary crude protein on growth, ammonia concentration and litter composition in broilers. Poult. Sci. 77: 1481-87.

Gatel F, Grosjean F (1992). Effect of protein content of the diet on nitrogen excretion by pigs. Livestock .Prod. Sci. 31: 132-147.

Hall LE, Shirley RB, Bakalli RI, Aggrey SE, Pesti GM., Edwards Jr. HM (2003). Power of two methods for the estimation of bone ash of broilers. Poult. Sci. 82: 414-418.

Holshemier JP, Veerkamp CH (1992). Effect of dietary energy, protein and lysine content on performance and yields of two strains of male broiler chicks, Poult. Sci. 71: 872-879.

Jacob JP, Blair R, Bennett DC, Scott TA, Newberr RC (1994). The effect of dietary protein and amino acid levels during the grower phase on nitrogen excretion of broiler chickens. In Proceedings of Canadian Animal Science Meetings, University of Saskatchewan, Saskatoon, SK, Canada. p. 309.

Marks HL, Pesti GM (1984). The roles of protein level and diet form in water consumption and abdominal fat pad deposition of broilers. Poult. Sci. 63: 1617-1625.

Mitchell RD, Edwards Jr. HM (1996). Additive effects of 1,25-dihydroxycholecalciferol and phytase on phytate phosphorus utilization and related parameters in broiler chickens. Poult. Sci. 75: 111-119.

NRC (1994). Nutrient Requirements of Poultry. 8th rev. ed. National Academy Press, Washington, DC. USA.

Paul JW, Barton PK, Kermode D, Scott TA (1996). Measuring ammonia emissions from poultry broilers fed balanced amino acid diets. Poult. Sci. 75(1): p. 26.

Plumstead PW, Leytem AB, Maguire RO, Spearrs JW, Kwanyuen P, Brake J (2008). Interaction of calcium and phytate in broiler diets. 1. Effects on apparent prececal digestibility and retention of Phosphorus. Poult. Sci. 81: 449-458.

Qian H, Kornegay ET, Denbow DM (1997). Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76: 37-46.

Reece N, Bates B, Lott BD (1979). Ammonia control in broiler houses. Poult. Sci. 58: 754-755.

Sebastian S, Touchbrun SP, Chavez AER, Lague PC (1996). Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance, and mineral utilization of broiler chickens. Poult. Sci. 75: 1516-23.

Sebastian S, Touchburn SP, Chavez AER, Lague PC (1997). Apparent Digestibility of Protein and Amino Acids in Broiler Chickens Fed a Corn-Soybean Diet Supplemented with Microbial Phytase. Poult. Sci. 76: 1760-1769.

Shafey TM, McDonlad MW, Pym RAE (1990). Effects of dietary calcium, available phosphorus and vitamin D on growth rate, food utilisation, plasma and bone constituents and calcium and phosphorus retention of commercial broiler strains. Br. Poult. Sci. 31: 587-602.

Smuts MCJ, Valk H, Elzing A, Keen A (1995). Effect of protein nutrition on ammonia emission from a cubicle house for dairy cattle. Livestock Prod. Sci. 44: 147-156.

Temim S, Chagneau AM, Guillaumin S, Michael J, Peresson R, Geraert PA, Tesseraud S (1999). Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens. Reprod. Nutr. Dev. 39: 145-156.

Temim S, Chagneau AM, Guillaumin S, Michael J, Peresson R, Tesseraud S (2000). Does excess dietary protein improve growth performance and carcass characteristics in heat-exposed chickens? Poult. Sci. 79: 312-317.

Ziaei N, Guy JH, Edwards SA, Blanchard P, Ward J, Feuerstein D (2006). Effect of reducing dietary protein and mineral content on growth performance, nutrient digestibility and bone strength of broiler chickens. Br. Poult. Sci.. 2(1): 13-14.

Ziaei N, Guy JH, Edwards SA, Blanchard P, Ward J, Feuerstein D (2008). Effect of reducing dietary mineral content on growth performance, water intake, excreta dry matter content and blood parameters of broilers. Br. Poult. Sci. 49: 195-201.

Zyla K, Koreleski J, Swiatkiewicz S, Wikiera A, Kujawski M, Piironnen J, Ledouxs DR (2000). Effects of phosphorolytic and cell wall-degrading enzymes on the performance of growing broilers fed wheat-based diets containing different calcium levels. Poult. Sci. 79: 66-76.