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GROWTH PERFORMANCE AND SOME SERUM, BONE AND FECAL PARAMETERS OF BROILERS FED WITH DIFFERENT LEVELS OF CALCIUM AND PHOSPHORUS

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Abstract: Aim of the study was to evaluate the effects of diet non-phytate phosphorus (P) and calcium (Ca) level on growth performance and some blood, bone and faeces parameters of broilers. A total of 480 one-day-old Ross 308 broilers were randomly allocated to 3 dietary treatments with 5 replicates for a 42-d study. Corn-soybean meal based diets were consisted of three different Ca and P concentrations for starter and grower periods. High, medium and low Ca and P levels in starter and grower periods were 1.05-0.49, 0.95-0.44, 0.85-0.41% and 0.87-0.42, 0.78-0.38, 0.69-0.34%, respectively. After the first 21-d feeding period, no differences were observed for feed intake (FI) but body weight (BW) and feed conversion ratio (FCR) were higher ($P<0.05$) in group fed 0.85-0.41% Ca and P. Diet with low Ca and P tended to increase final body weight of the chicks at 42 days old. No differences were observed for FI, FCR and mortality among the treatments. Different Ca and P levels had no effect on internal organ weights ($P>0.05$). Fecal ash, tibia and sternum weight and sternum ash were not affected from the Ca and P concentrations but tibia ash was lower ($P<0.05$) in group having low concentration of Ca and P. Decreasing levels of Ca and P had a negative effect on relative breast meat weight ($P<0.05$). There were no significant differences in Ca, P concentrations and aspartate transaminase (AST), alanine transaminase (ALT) activities in blood serum ($P>0.05$). However, alkaline phosphatase (ALP) enzyme activity was higher ($P<0.05$) in group having medium levels of Ca and P. Based on the data, it can be concluded that 8.5 g/kg Ca and 4.1 g/kg P can be used for starter period without any deterioration on growth performance.

Keywords: Broiler, Ca-P level, Growth performance, Serum parameters

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1. Introduction

The requirement of calcium (Ca) and phosphorus (P) of broilers has been the subject of research for decades. Studies conducted to determine Ca and P requirements of broilers has shown that demand of these minerals have changed with the development of genetic, nutrition and environmental conditions. Recent studies have reported that not only Ca and P concentration but also Ca and P ratio is essential for bone development and growth performance (Driver et al., 2005; Rama Rao et al., 2006; Selle et al., 2009; Han et al., 2016). The majority of P in plant source is present in the form of phytate, 28.2% of the total P is called nonphytate P (NPP) (Ravindran et al., 1995). Due to poor solubility, phytate is poorly absorbed by broilers (Wilkinson et al., 2014a). Plants are insufficient to provide the requirements of Ca and NPP for broilers therefore, some inorganic ingredient such as dicalcium phosphate (DCP) and limestone are added to broilers feeds. High acid binding capacity of the limestone leads to poor solubility of proteins and P in the gizzard (Walk et al., 2012). Decreasing Ca concentration of feed have led to develop P utilization but excess Ca decreased P concentration of bone ash (Letorneau-Montminy et al., 2008).

Phosphorus retention may be developed with the early restriction of Ca and NPP. This improvement arises from the adaptation ability of broilers to early restrictions and adaptation also may improve late period performance and P retention (Yan et al., 2005). Some studies have suggested that development of P utilization may be carried out with the arrangements of Ca concentration (Driver et al., 2005; Letourneau-Montminy et al., 2010; Roussea et al., 2012). Low Ca and narrow Ca:P ratios have improved P utilization (Qian et al., 1997; Liu et al., 1998; Brady et al., 2002; Selle et al., 2009). As stated before, diets having sufficient Ca and low NPP can cause similar performance values with diets having relatively high concentration of Ca and NPP (Rama Rao et al., 2006). It is generally accepted that increase of Ca in the diet may increase bone ash content (Driver et al., 2005). However, excess dietary Ca may have a negative effect on birds' performance (Sebastian et al., 1996).

Development of P utilization and determining sufficient concentrations of dietary Ca and NPP requirements for broilers should help to reduce the amount of dietary nonphytate phoshporus (NPP) supplementation needed and also reduce potential environmental pollution (Delezie et al., 2012). Broiler diets are typically formulated to involve between 8.0 and 10.0 g/kg Ca and 4.0 and 5.0 g/kg NPP but advancements in the birds and nutritional strategies indicate that old suggestion cannot be applied at the present time.

The present study was designed to investigate the effect of three different Ca and P levels for both starter and finisher periods on growth performance, bone development with some serum and fecal parameters.

2. Materials and Methods

2.1. Birds and Housing

Commercial feather-sexed 480 one-day-old Ross 308 broilers were randomly allocated to 3 dietary treatments with 5 replicates for a 42-d study in June 2016. Experiment was designed with completely randomized plot design. Chicks were kept at a temperature of 33°C for first day and afterwards this was gradually decreased to 22 °C and between 22 and 42 days 22 °C was maintained. Lightning regime of the study consisted of 23L:1D for 42 days. On day 10 and 14 birds were vaccinated against infectus bursal disease and Newcastle disease, respectively, through drinking water. Birds were checked four times in a day and mortalities were recorded. Feeding and husbandry conditions, except the Ca and P levels, were all same with the company, producing the genotypes.

2.2. Diets and Experimental Procedures

Commercial corn-soybean meal based starter and grower diets were consisted of three different Ca and P concentrations. High, standard and low Ca and P levels in starter and grower periods were presented in Table 1. Ca and P levels were reduced by degrees ranging from 10 to 11% to constitute experimental diets as presented Table 2.

Table 1. Experimental design

	Diet Ca -P levels (%)			
Groups	Starter diet		Grower diet	
High	1.05	0.49	0.87	0.42
Standard	0.95	0.44	0.78	0.38
Low	0.85	0.41	0.69	0.34

Table 2. Ingredients and chemical composition of starter and grower diets

Ingredients (g/kg)	Starter diet			Grower diet		
	High	Std.	Low	High	Std.	Low
Corn	575	571.46	577.96	610.71	612.81	615.7
Soybean Meal (48%)	360	362.11	360.83	314.71	320	320
Vegetable oil	21.58	29.09	27.04	35	35	35
Limestone	14	14	12.3	12.22	11	10
Dicalcium phosphate	18.5	15.5	13.5	14.85	12.38	10.5
Sodium chloride	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin-mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
DL-methionine	2.84	2.84	2.84	2.48	2.48	2.48
Lysine	2.5	2.5	2.5	2.5	2.5	2.5
Chemical composition(%)						
Dry matter	88.40	88.21	88.33	88.13	88.67	88.40
Crude protein	21.91	21.85	21.87	19.85	19.91	19.88
Ether extract	5.14	5.31	5.13	5.72	5.72	5.72
Crude fiber	3.63	3.60	3.61	3.42	3.46	3.47
Crude ash	5.84	5.80	5.71	5.75	5.41	5.14
Calcium	1.05	0.95	0.85	0.87	0.78	0.69
AME (kcal/kg)	3050	3050	3050	3200	3200	3200
Calculated composition(%)						
Available phosphorus	0.49	0.44	0.41	0.42	0.38	0.34
Lysine	1.40	1.40	1.40	1.27	1.27	1.27
Methionine+cystine	1.00	1.00	1.00	0.90	0.90	0.90

[1]Vitamin A, 12000 IU; Vitamin D_3, 2400 IU; Vitamin E, 30 IU; Vitamin K_3, 2.5 mg; Vitamin B_1, 3.0 mg; Vitamin B_2, 7 mg; Nicotin amid, 40 mg; Calcium D-pantothenate, 8.0 mg; Vitamin B_6, 4.0 mg; Vitamin B_{12}, 0.015 mg; Folic acid, 1 mg; D-biotine, 0.045 mg; Vitamin C, 50 mg; Chlorine chloride, 125 mg., Mn, 80 mg; Fe, 40 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.1 mg; I, 0.4 mg; Se, 0.15 mg

A total of 480 one-day-old Ross 308 broilers were randomly allocated to 3 dietary treatments with 5 replicates for a 42-d study. Bird density was 13 chicks per square meter. Each pen was equipped with one hanging feeder. Feed, in mash form, and water were provided to birds as ad libitum throughout the trial. The chemical compositions of diets were determined according to the methods of AOAC (1990). Calculation of metabolic energy was done according to the Turkish Standards Institute (TSE) (1991). Starter and grower diets were offered to birds during days 1 to 21 and from days 22 to 42, respectively.

Feed intake (FI) and BW were recorded weekly. All birds were weighed individually at days 0, 7, 14, 21, 28, 35 and 42 to evaluate body weights. Feed intake was determined for each replicate at these days. Calculation of FCR was made via ratios of FI to BW gain on replicate basis. Mortalities were considered while FCR was calculating. At day 42, ten birds (1 male and 1 female from each replicate) representing average body weights of the group (±5%) were slaughtered from each group. Carcass yield, relative weight of the carcass cuts and internal organ weight were determined. Left tibias and sternums were individually removed and cleaned to determine bone weight and bone ash contents. Bones were waited at room temperature for 6 hours before analysis started. Each tibia and sternum were broken into small pieces, weighed and ashed at 600°C for 12 h.

At day 39, 40 and 41 about 200g faeces from each replicate were collected at the same time every day. Fecal samples were cleaned from all residues. Fecal samples were waited at 105 °C to determine dry matter and thereafter these samples were ashed at 600°C for 6 h to determine ash content.

Blood samples were collected by cardiac puncture and saved into empty collection tubes in order to obtain serum. Serum were separated by centrifugation at 1800xg and promptly analyzed. Serum Ca, P concentrations (Roche Diagnostics), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) enzyme activities were determined with a spectrometers using commercial kits (Olympus AU-600).

2.3. Statistical Analysis

Data were analyzed by ANOVA using the Minitab 16 package program. An arc-sin transformation was applied to the percentage values (i.e. mortality) before testing for differences. Significant differences among means of treatments were determined by Duncan's multiple range test with 5% probability.

3. Results

Data regarding performance during the overall growth period (1 to 42 d) are presented in Table 3. Calcium and P levels had no significant effect on FI (P>0.05). Low Ca and P levels tended to decrease FI at 42 days of age. Body weight of 14, 21 and 28 days of age were effected from Ca and P levels (P<0.05), the highest body weight was obtained from group fed low Ca and P. Final body weight was not effected from Ca and P levels (P>0.05). Different Ca and P had no significant effect on mortality (P>0.05). Feed conversion ratios of 14, 21, 28 and 35 days of age had a significant difference (P<0.05) and the best FCR values were obtained from the group which was fed with low Ca and P.

Table 3. Effects of different Ca and P levels on feed intake (FI), body weight (BW) and feed conversion ratios (FCR)

Diet	FI, g					
	Day					
	7	14	21	28	35	42
High	139.2	529.0	1130.9	2031.0	3156.1	4512.3
Standard	139.3	526.0	1124.6	2050.0	3201.3	4598.4
Low	140.9	543.7	1144.0	2062.8	3163.1	4455.5
SEM	4.17	8,24	15.73	29.56	40.70	60.63
P value	0.954	0.319	0.693	0.787	0.751	0.341
	BW, g					
High	154.2	425.7^{b}	820.2^{ab}	1325.3^{b}	1925.5	2589.5
Standard	158.4	414.7^{b}	789.3^{b}	1313.6^{b}	1936.3	2626.3
Low	161.7	447.0^{a}	851.0^{a}	1394.3^{a}	1983.0	2762.0
SEM	2.17	5.01	10.00	16.13	27,80	54,53
P value	0.099	0.003	0.004	0.009	0.376	0.139
	FCR					
High	1.28	1.39^{b}	1.46^{b}	1.58^{ab}	1.67^{ab}	1.78
Standard	1.23	1.42^{a}	1.51^{a}	1.61^{a}	1.69^{a}	1.78
Low	1.21	1.35^{c}	1.42^{c}	1.52^{b}	1.63^{b}	1.71
SEM[1]	0.02	0.01	0.01	0.01	0.01	0.02
P value	0.440	<0.001	0.007	0.009	0.041	0.112

$^{a, b,c}$ Values within a column not sharing the same superscript are different at P<0.05, SEM= standard error of means.

Data regarding carcass weight, carcass yield and carcass parts are presented in Table 4. Calcium and P levels had no significant effect on carcass yield, thigh and wing weight (P>0.05). However, breast weight was effected from Ca and P levels (P<0.05). Low Ca and P levels caused the lowest breast weight. There was no significant difference between groups in that internal organ weights (P>0.05). Proportional internal organ weights were presented in Table 5.

Serum Ca and P concentrations and ALP, ALT and AST enzyme activity values were presented in Table 6. There were no treatment differences in serum parameters (P>0.05), with the exception of ALP enzyme activity. Medium Ca and P levels caused the highest ALP enzyme activity (P<0.05). There were no treatment differences in faeces ash and dry matter content (P>0.05).

The effects of different Ca and P levels on bone development are presented in Table 7. There were no significant differences in tibia and sternum weights (P>0.05).

Table 4. Carcass weight(CW), carcass yield(CY), proportional and real thigh, breast and wing weight of broilers fed different levels of Ca and P

	Low	Standard	High	SEM	P value
CY, %	76.8	76.5	75.7	0.51	0.355
Breast, %	36.3[b]	38.3[a]	38.3[a]	0.45	0.008
Thigh, %	27.5	26.8	26.4	0.43	0.296
Wing, %	10.0	10.0	9.9	0.20	0.899
CW, g	2125.4	2092.4	2059.1	84.66	0.467
Breast, g	773.7	803.9	790.3	56.16	0.493
Thigh, g	584.4	563.0	545.9	46.41	0.197
Wing, g	213.3	209.7	204.2	13.58	0.335

[a, b] Values within a column not sharing the same superscript are different at P<0.05, SEM= standard error of means.

Table 5. Effects of different Ca and P levels on proportional gizzard, liver, spleen, pancreas and intestine weights (Weight of organ/Body weight)

Diet	Gizzard	Liver	Spleen	Pancreas	Intestine
High	1.612	1.965	0.106	0.245	2.295
Medium	1.503	2.145	0.098	0.256	2.173
Low	1.515	1.864	0.108	0.230	2.438
SEM	0.04	0.08	0.01	0.02	0.10
P value	0.215	0.070	0.789	0.656	0.235

SEM= standard error of means.

Table 6. Effect of different Ca and P levels on serum calcium-phosphorus concentration and aspartate transaminase, alanine transaminase, alkaline phosphatase enzyme activity

Diet	Ca (mg/dL)	P (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
High	11.549	7.203	408.8	2.812	1415[b]
Medium	11.312	7.172	350.7	1.984	3199[a]
Low	11.345	7.054	378.2	1.912	1692[b]
SEM	0.17	0.17	32.73	0.40	253.60
P value	0.619	0.813	0.486	0.142	<0.001

[a,b]Values within a column not sharing the same superscript are different at P<0.05, SEM= standard error of means, AST= aspartate transaminase, ALT= alkaline transaminase, ALP= alkaline phosphatase.

Table 7. Effects of different Ca and P levels on tibia and sternum weights and ash contents

Diet	Tibia		Sternum	
	Weight, g	Ash, %	Weight, g	Ash, %
High	8.205	42.29[a]	3.656	40.71
Medium	8.518	41.67[a]	3.888	40.01
Low	8.424	37.52[b]	3.779	40.99
SEM	0.371	0.960	0.148	0.876
P value	0.835	0.008	0.563	0.737

[a, b] Values within a column not sharing the same superscript are different at P<0.05, SEM= standard error of means.

However, ash content of the tibia had a significant difference between groups (P<0.05). Tibia ash content decreased in proportion with Ca and P level. The lowest tibia ash content was obtained from group fed with low Ca and P. There was no significant difference in sternum ash content (P>0.05).

4. Discussion

It was stated before that not only the amount of the Ca and P in diet but also the ratio between these minerals are essential for performance and bone development of broilers (Driver et al., 2005; Han et al., 2016; Rama Rao et al., 2006; Selle et al., 2009). Amounts of the Ca and P were changed but the ratio between them was tried to be arranged similar in this experiment. It was known that reducing diet Ca and P level can let to obtain the similar performance values if the ratio between them is protected (Rama Rao et al., 2006). Feed intake is related to diet P level and high P and medium Ca levels were said to cause the best feed intake for broilers. In this experiment low starter and grower feeds containing, %0.85-0.69 Ca and 0.41-0.34 P respectively. These levels are similar with the stated values for the best performance and feed intake (Driver et al., 2005; Rama Rao et al., 2006; Hamdi et al., 2015; Rousseau et al., 2016). The highest body weights were obtained from the low Ca and P group at 14, 21 and 28 days of age. Delezie et al. (2012) achieved the highest body weight values with the similar Ca and P levels with this experiment at 14, 21 and 28 days of age. Hamdi et al. (2015) expressed that %0.90 Ca and 0.45 P provided the highest body weight at 14 days of age. Phosphorus digestibility is related to the diet Ca and P level and ratio between these minerals and this mechanism originates from the antagonistic relation in small intestine (Günther and al-Masri, 1988; Al-Masri 1995; Driver et al., 2005; Selle, 2009). Therefore, improvement of the growth performance can be a result of reducing Ca level, it was stated that, low Ca level led to increase availability of some other nutrients (Wilkinson et al., 2014b). Additionally, the results for feed conversion ratio are similar to the findings of earlier studies (Driver et al., 2005; Rama Rao et al., 2006; Delezie et al., 2015; Han et al., 2016).

No differences were observed in carcass yield and carcass parts with the exception of proportional breast weight. Birds fed low Ca and P had the lowest breast proportion of carcass. Age, genotype, sex are the most effective factors on carcass part weights but according to some results genotype and feed composition are both effective on carcass parts and weights (Corzo et al., 2005).

Viveros et al. (2002) determined that different Ca and P levels had a significant effect on internal organ weights. Our results contradict with this earlier study but it is possible to explain this contradiction with the variation of Ca and P level.

Increasing ALP enzyme activity can be explained with the osteoblast activity which is high in young, growing birds and birds, having bone deteriorations. Although it was stated that intestine isoenzyme has the greatest effect on the ALP activity (Campbel and Coles, 1986), ALP is rarity in liver tissue but liver problems lead to increase the activity of it (Zantop, 1997). In some earlier experiment, it was stated that ALP enzyme activity increased when the Ca and P level was inadequate in diet (Rama Rao et al., 2006). Low P can lead to increase serum Ca concentration and increased Ca concentration repress the secretion of parathyroid hormone. Prohibitive effect of parathyroid hormone on phosphate absorption and disposal of Ca by urine decreased (Viveros, 2002). Therefore, according to obtained ALP activity values of this study, it can be concluded that there were no Ca and P deficiency in groups.

ALT activity of serum is slightly found in all tissues of birds (Bogin and Israeli, 1976). However, ALT activity generally increases with the tissue injuries (Zantop, 1997). Unlike mammals, AST is not a liver specific parameter. Activity of AST can increase with a problem in muscle tissues or liver tissue (Lewandowski and Harrison, 1986). Therefore, it can be said that any Ca and P levels in this experiment had a tissue injury.

Low diet P level did not affect serum Ca concentration in this study. Serum Ca and P concentration results coincide with a number of earlier studies (Sebastian et al., 1996; Fernandes et al., 1999; Viveros et al., 2002; Kheiri and Rahmani, 2006).

Many investigations have shown that decreasing diet Ca and P level has led to decrease tibia ash content (Onyango et al., 2003; Rama Rao et al., 2006; Adamu et al., 2011; Mello et al., 2012; Naves et al., 2014; Wilkinson et al., 2014a; Delezie et al., 2015; Hamdi et al., 2015; Han et al., 2016; Rousseau et al., 2016). Imbalance of the diet Ca:P ratio also had a negative effect on ash content of tibia (Driver et al., 2005; Delezie et al., 2012; Rousseau et al., 2016). Decreasing diet Ca and P level had a negative effect on tibia ash content in the present study.

5. Conclusion

From the data of the current experiment, it can be concluded that 0.85 g/kg Ca and 0.41 g/kg P can be used for starter period without any deterioration on growth performance. Gradually increasing cost of DCP, decreasing world P reserves, environmental pollution arising from fecal P and economic reasons for broiler sector must encourage researchers to try finding ways to decrease P level in broiler diets.

Facilities of decreasing P in broiler diets in view of the fact that animal welfare and bone problems with or without phytase enzyme supplementation must be investigated. Relation between serum and bone mineral contents and serum enzyme activity should be a focused subject.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	A.A.	Z.K.
C	50	50
D	50	50
S		100
DCP	100	
DAI	50	50
L	100	
W	100	
CR	50	50
SR	100	

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because this study conducted before 2020.

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