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Effects of Lower Dietary Calcium and Phosphorus on Growth Performance and Bone Mineralizatıon of Broilers

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ABSTRACT: This experiment was carried out to investigate the effects of reduced dietary calcium (Ca) and non-phytate-phosphorus (NPP) levels at 2:1 constant ratio on performance, carcase parameters and bone mineralization of Ross-308 broilers. A total of 11400 one-day-old chicks were randomly allocated to five dietary treatments with twelve replicates and were fed starter diets including either recommended (control treatment, T1) or a 6.25% lower level of both Ca and NPP according to breeder's recommendations during starter period (SP). After SP, control treatment (T1) continued to be provided through diets containing 0.87 and 0.78 % Ca for grower (GP) and finisher periods (FP), respectively, while 6.25 % reduced group was divided into 4 dietary treatments regarding extent of decrease in Ca and NPP levels and provided by diets containing 0.79;0.65 (T2), 0.79;0.60 (T3), 0.75;0.65 (T4), and 0.75;0.60 (T5) % Ca for GP and FP, respectively. After SP, moderate and even substantial reduction of Ca and NPP significantly decreased feed intake (FI) (quadratic, $P<0.05$), but obtained similar body weight gain (BWG) and FCR compared to the control ($P>0.05$). On the other hand, reduced dietary Ca through T1 to T5 significantly decreased the total Ca and NPP intake of broilers (linear and quadratic, $P<0.01$), and accompanied to significant linear and quadratic relationship between Ca intake and FCR and BWG respectively. It can be concluded that reducing Ca level down to 0.75 and 0.60 % in grower and finisher phase, respectively are possible without compromising the growth performance and bone mineralization of modern broilers.

***Keywords*: broiler; bone mineralization; calcium and phosphorus level; performance**

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INTRODUCTION

Ca (calcium) and P (phosphorus) are two main macro minerals retained in the body, and essential for bone formation, skeletal muscle development, enzyme activity, acid-base balance, and metabolism (Underwood and Suttle, 1999). An excessive or deficient level of Ca or P in the diet often leads to deficiency or excess of others, which is due to the interactions between the two minerals affecting Ca and P availability and endogenous excretion (Al Masri, 1995). Therefore, for nutritionist, their supplementation is considered together, and the ratio between Ca and P is tried to keep constant around 2:1. This ratio is mentioned as a critical point in Ca and P nutrition of broilers to obtain better utilization of the minerals. (Mello et al., 2012; Kiani and Tahiri, 2020).

Broiler diets are mainly supplemented with inorganic Ca and P sources such as limestone and dicalcium phosphate, respectively, to meet the requirements, since broiler diets are mostly based on feedstuffs of plant origin. However, these ingredients usually consist of the high amount of P in the phytate form (60 to 80%), which is mainly unavailable to be used for broilers (Applegate and Angel, 2008; Cowieson et al., 2016), and this low availability results in the high level of P excretion via manure and environmental severe concern. Besides, global feed phosphate resources are limited and an expensive nutrient in broiler diets (approx. 150-300 $ vs. 15-30 $ per metric tons of Ca). Therefore, more effort that is tremendous has been made to improve and assure maximum P availability in the diet as well as to avoid an excess of P in poultry diets and excreta (Hamdi et al., 2015). Despite plenty of research have been performed on Ca and P during the last decades, and their requirements in broiler chicks are considered settled, and a done deal, more recent studies (Li et al., 2017; Valable et al., 2018; Kiani and Tahiri, 2020; Ceylan et al., 2020) has dramatically changed the perception of the actual Ca and P needs, which can be attributed to the change in growth potential and body composition (percentage of breast meat) of broilers. The ratio between soft tissues and bones (more soft tissues - fewer bones) might also be changed, which consequently may affect the Ca and P requirement of modern fast-growing broilers (van Krimpen et al., 2013). Slight reduction in Ca and NPP requirements of Ross-308 broilers by the breeding company, approximately 6.3 % and 4.4 %, respectively in 2014 compared to 2009, may be attributed to the mentioned changes; even the latest edition of nutrient specifications in 2019 has no difference.

Besides, not only above mentioned assumption on change for Ca and P requirement, but also the quantity of Ca level in broiler feed is also critical to pay attention because of the acid-neutralizing capacity of limestone and harmful effects of excess calcium. Reducing dietary P level is possible if the Ca level is simultaneously decreased (Rousseau et al., 2016) due to a lower propensity for Ca-phosphate and Ca-phytate complexes in the digestive tract. Excessive Ca in poultry diets not only has the capacity to interfere with digestion and absorption of micro minerals, but it also has the potential to interact with inorganic P in the gut as it has claimed by Hamdi et al. (2015) that lower dietary Ca has potential to improve P and micro minerals utilization for broilers. Moreover, limestone, widely used to supply Ca in diet, has high acid-binding capacity related to not only decrease in P utilization but also protein solubility in the gizzard and may negatively affect both P and nitrogen (N) utilization (Selle et al., 2009; Walk et al., 2012). Many studies also emphasized that Ca supplied over requirement may cause decreased dietary energy utilization and reduction in growth performance and feed efficiency via negatively affects the process of digestion by owing to the formation of insoluble salts with dietary fatty acids (Delezie et al., 2012; Li et al., 2015).

The two above-mentioned aspects have thus led poultry nutritionists to focus on conducting broiler experiments to evaluate the reduced dietary Ca levels with different approaches as the severity of reduction is critical for broiler growth and bone mineralization through the whole fattening period. A strategy for lowering dietary Ca and P needs to consider the structural organization of broiler development with age. Sanchez-Rodriguez et al. (2019) identified that during the earliest stages of growth (first 2 wk), the proper mineralization and structuring of bone are compromised, and specific actions need to be done at an early age. Mello et al. (2012) thus reported that the NPP requirement of broilers at the starter phase could be high, but this amount may be reduced during the growth and finishing phase. Recently Valable et al. (2018) also showed that after the standard starter period, 0.60 and 0.48 % dietary Ca for the grower-finisher phase, respectively, were enough to maintain performance, but not for tibia mineralization. But Ceylan et al. (2020) did not observe any harmful effects with a bit higher Ca level, 0.75 and 0.60 %, during the grower and finisher phase, respectively, on growth performance and tibia mineralization. Hence, Ca and P lowering strategies, especially after the starter period, may not only prevent structural development of bones but also contribute to reducing the excretion of these minerals via manure as most of the feed consumed after ten days, over 90 %.

Today, modern broilers Ca and P requirements alteration is on agenda because of the above-mentioned subjects, but how its implementation is still question because there is no consensus for which growth periods and at what level are controversial. For this purpose, we evaluated the hypothesis that a severe reduction in dietary Ca and NPP level by keeping the constant 2:1 ratio could be implemented during the grower and finisher phase, after a slight decrease in the starter phase without compromising body growth potential and bone mineralization of broiler chicks.

MATERIALS AND METHODS

Ethical Statement

This study was approved by the University Animal Experiments Local Ethics Committee (Approval no: 207-02-28).

Bird management and sample collection

The experiment was conducted in the broiler research development center, located in the Bolu region in Turkey. A total of 11400 one-day-old Ross 308 broiler chicks were weighed at the beginning of the study and randomly allocated to 60-floor pens (6.5x2m each) containing 190 chicks (95 male, 95 female), each equipped nipple drinkers and computer-controlled plastic hanging feeder. Wood shavings were used as litter material in the floor pens. Chicks were hold wood shavings equipped floor pens and allowed ad libitum access to water and feed (crumble and pellet form for starter and remaining period, respectively) through the experiment. The research unit temperature was set to 33˚C for the first three days, then decreased to 23˚C gradually till 21 d, and then maintained at this temperature until the end of the experiment via automatic climate systems. All chicks were cared for according to the guidelines in the Ross-Broiler Handbook through the investigation (Aviagen 2018).

Experimental diets

Before the experiment, main ingredients and Ca sources were analyzed and then the experiment was arranged in a completely randomized block design with five dietary treatments after the starter phase. During the starter period, broiler chicks were fed the diet including either recommended (control treatment, T1) or a 6.25% lower level of both Ca and NPP according to the breeder's recommendations during 0-10 days (Aviagen 2019). After the starter period (SP), control treatment (T1) continued to be provided through diets containing 0.87 and 0.78 % Ca for grower (GP) and finisher periods (FP), respectively, while 6.25% reduced group was divided into four dietary treatments regarding extent of decrease in Ca and NPP levels and provided by diets containing 0.79;0.65 (T2), 0.79;0.60 (T3), 0.75;0.65 (T4), and 0.75;0.60 (T5) % Ca for GP and FP, respectively.

Due to the same extent of decrease in Ca levels of Ca-reduced treatments for the starter period, T1 had 12 replicates as there were 48 replicates for Ca-reduced groups for 0-10 days, and then each treatment had twelve replicates for remaining periods. Experimental diets were formulated based on corn-soybean meal based, for starter (0-10 days), grower (11-24 days), finisher (25-37 days) phases, and then identical diets only without anticoccidial introduced during the last three days of the 40-day experiment. Experimental diets were formulated to be isonitrogenous for each periods and to meet or exceed of broiler chickens demands except for Ca and NPP requirements as above-mentioned. Experimental diets formulation and analysis results were shown in Table 1. Analysed results of experimental diets were in close agreement with calculated values. Experiment feeds were produced in a feed mill of Beypilic. While, micro-ingredients were weighed by a micro dosage system, the rest of the ingredients were taken from the system silos into mixer. After that, mineral additives were weighed, at batched properly on an outside scale, for each treatment, and then all components were collected into six tons capacity mixer. After all materials were put together into the mixer, all feeds were mixed for 210 sec and then pelleted by conditioning at 75˚C.

Measurement

Throughout the experiment, chicks were weighed per pen basis at 0, 10 and 40 days and Feed intake (FI) was recorded for each growing period: 0 to 10, 11 to 40, and 0 to 40 days. In line with this, feed conversion ratio (FCR) was calculated for 0-10, 11-40, and 0-40 days using FI and body weight gain (BWG). Mortality was recorded by daily. When mortality occurred, chick was weighed and removed. Mortality rate was calculated for each growing periods as a cumulative percentage for calculation of FCR (Sikandar et al. 2020). Growth performance data were given and

Table 1. Ingredient and nutrient composition of experimental basal diets (% as fed).

	Starter (0-10 day)		Grower (11-24 day)			Finisher[1] (25-40 day)		
Ca,%	0.96	0.90	0.87	0.79	0.75	0.78	0.65	0.60
NPP,%	0.48	0.45	0.44	0.65	0.38	0.39	0.33	0.30
Ingredients								
Maize	46.34	46.88	48.79	49.35	49.63	53.38	54.39	54.75
Wheat	5.00	5.00	6.00	6.00	6.00	7.00	7.00	7.00
Soybean meal	19.28	19.98	14.49	14.30	14.20	9.69	10.11	10.14
Sunflower meal	3.50	3.50	4.50	4.50	4.50	5.00	5.00	5.00
Full-fat soybean	15.89	14.87	15.22	15.34	15.39	12.40	11.64	11.53
Maize gluten meal	1.50	1.50	1.00	1.00	1.00	1.00	1.00	1.00
Poultry by-product meal	3.00	3.00	4.00	4.00	4.00	5.00	5.00	5.00
Soybean oil	1.40	1.40	2.50	2.30	2.20	3.50	3.30	3.20
Dicalcium phosphate	1.04	0.87	0.95	0.72	0.61	0.72	0.47	0.33
Monocalcium phosphate	0.70	0.70	0.50	0.50	0.50	0.40	0.30	0.30
Limestone	0.79	0.74	0.67	0.61	0.57	0.58	0.46	0.42
Sodium bicarbonate	0.18	0.18	0.15	0.15	0.15	0.14	0.14	0.14
Salt	0.23	0.23	0.22	0.22	0.22	0.20	0.20	0.20
DL-Methionine	0.16	0.16	0.15	0.15	0.15	0.14	0.14	0.14
Methionine hydroxy analogue	0.20	0.20	0.15	0.15	0.15	0.10	0.10	0.10
L-Lysine	0.39	0.39	0.34	0.34	0.34	0.36	0.36	0.36
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.05	0.05	0.40	0.40	0.40	0.04	0.04	0.04
Anticoccidial	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Calculated and analysed nutrient composition[4]								
ME, Mj/kg	12.56	12.56	12.98	12.98	12.98	13.40	13.40	13.40
Crude protein (%)	23.00(22.90)	23.00(23.05)	21.50(21.60)	21.50(21.60)	21.50(21.45)	19.50(19.30)	19.50(19.45)	19.50(19.50)
Crude ash (%)	6.53(6.50)	6.32(6.25)	6.00(5.95)	5.72(5.75)	5.58(5.55)	5.38(5.40)	4.92(4.95)	4.74(4.65)
Ca (%)	0.96(0.98)	0.90(0.92)	0.87(0.90)	0.79(0.81)	0.75(0.74)	0.78(0.75)	0.65(0.67)	0.60(0.59)
Total P (%)	0.75(0.78)	0.73(0.74)	0.70(0.68)	0.66(0.66)	0.64(0.65)	0.63(0.65)	0.57(0.58)	0.55(0.54)
NPP (%)	0.48	0.45	0.44	0.40	0.38	0.39	0.33	0.30
Lys (%)	1.44	1.44	1.29	1.29	1.29	1.15	1.15	1.15
Met+Cys (%)	1.08	1.08	0.99	0.99	0.99	0.90	0.90	0.90

[1] Finisher diets was used up to end of 37 day of age, then same diets only without anticoccidial introduced through 3 day.
[2] Supplied per kg diet: 10,000 IU vitamin A, 4500 IU vitamin D3, 65mg vitamin E, 2.8 mg vitamin B1, 6.5 mg vitamin B2, 3.2 mg vitamin B6, 0.017 mg vitamin B12, 3.5 mg vitamin K3, 18mg pantothenic acid, 55 mg niacin, 0.18 mg biotin,1.9 mg folic acid
[3] Supplied per kg diet: 20 mg Fe, 16 mg Cu, 110 mg Zn, 120 mg Mn, 1.25 mg I, 0.9 mg Co, 0.3 mg Se.
[4] Values in parenthesis represent analysed contents of nutrients.

evaluated over the whole feeding period. European Production Efficiency (EPEF) was also calculated at the end of the experiment. Ca and NPP intake were calculated from FI and presented as gram and % of BWG. The relationship between the Ca intake, and FCR or BWG presented as a regression analysis. At the end of the experiment, feed was removed six hour before processing and two chicks per pen (one male, one female), close to the average pen weight, were selected for further investigation. Before the slaughter, each bird was weighed and leg-banded for identification then exsanguinated by cutting the jugular vein, allowed to bleed for approximately 2 min, scalded for 30 sec, and de-feathered. Afterward, following viscera and abdominal fat removing, carcass, drumsticks and breast meat were weighed and calculated as a fraction of individual live body weight. In experiment diets, crude ash was determined by placing samples in a burning oven at 550°C for 12 h and, crude protein (Nx6.25) analysis was determined by using the Kjeldahl method. Ca was resolved atomic absorption spectrophotometrically and, total P was determined by the colorimetric vanamolybdate procedure (AOAC, 2005). For ash and P analysis in bone, left tibias from each replicate were removed and kept at -20˚C before performed analysis then; tibias were defatted for 48 h in ethyl alcohol followed by a 48 h extraction in ethyl ether. They were then dried for 12 h at 110°C and then ashed overnight at 600°C in a muffle furnace and tibia ash, as the percentage of tibia dry matter weight was determined. The ash for each tibia was then further analyzed for phosphorus (AOAC, 2005).

Statistical Analyses

Data obtained in the present study were subjected to statistical analysis using the GLM procedure of Minitab 18 in a randomized complete block design. There were twelve replicates for all measurements except for the starter period parameters. At that period, two groups were analyzed with twelve replicates for control and forty-eight replicates for Ca reduction group with T test. For the remaining parameters, the linear and quadratic contrasts were used to compare the effects of decreasing dietary Ca and NPP levels. Probability values of P<0.05 were considered significant, and means were separated using the Tukey HSD test. The mortality data were subjected to a chi-square test.

RESULTS

Results of the present experiment on growth performance (BWG, FCR, FI, mortality and EPEF) in broilers for investigated periods were given in Table 2. As shown in Table 2, BWG, FI and FCR were not significantly affected (P>0.05) by the treatments at starter period. Only in that period, mortality rate was significantly declined (P<0.05) by the reducing Ca level. In other words, Ca reduction from 0.96 to 0.90 % did not negatively affect the growth performance of broiler chicks. During both 11-40 and 0-40 days, only FI was significantly decreased with the reduced dietary Ca and NPP level in quadratic manner (P<0.01). Dietary reduction of Ca at all levels, T2-T5 diets, down to 0.75 and 0.60 % through GP and FP, respectively resulted in similar BWG, FCR, mortality rate and EPEF compared to the control treatment (T1) (P>0.05). Although EPEF and FCR results of T5 birds received the lowest Ca and NPP diets at both GP and FP was the best numerical value, there was no significant linear or quadratic effect (P>0.05).

Results of carcase parameters and tibia bone characteristics obtained in the experiment were given in Table 3. As shown in Table 3, there was no significant linear or quadratic effect (P>0.05) with the reduction

Table 2. Effects of different dietary Ca and NPP reduction at different periods on performance in broiler chickens.

Treatments	0-10 days				11-40 days				0-40 days				
	BWG g/bird	FI g/bird	FCR g feed/g gain	Mortality %	BWG g/bird	FI g/bird	FCR g feed/g gain	Mortality %	BWG g/bird	FI g/bird	FCR g feed/g gain	Mortality %	EPEF
T1	254.89	320.70	1.26	1.56	2139.25	3958.92 a	1.85	4.88	2394.14	4281.37 a	1.79	6.44	311.28
T2	255.93	321.87	1.26	0.92	2148.46	3887.13 bc	1.81	3.94	2400.08	4210.66 b	1.76	4.69	324.19
T3					2094.75	3847.11 c	1.84	4.94	2349.63	4165.93 c	1.77	5.75	310.49
T4					2143.33	3891.72 b	1.82	5.44	2396.01	4213.85 b	1.76	6.38	317.04
T5					2147.76	3902.57 b	1.82	3.88	2412.28	4225.43 b	1.75	5.06	324.88
SEM	3.804	2.071	0.021	0.205	21.045	18.277	0.013	0.663	21.045	18.372	0.012	0.727	5.941
P-linear[1]	0.789	0.581	0.937	0.014	0.862	0.114	0.186	0.816	0.646	0.117	0.087	0.653	0.300
P-quadratic					0.254	0.001	0.506	0.496	0.156	0.001	0.682	0.875	0.660

BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; EPEF: European Production Efficiency.
T1: 0.96 %, 0.87 %, 0.78 % Ca level; T2: 0.90 %, 0.79 %, 0.65 % Ca level; T3: 0.90 %, 0.79 %, 0.60 % Ca level; T4: 0.90 %, 0.75 %, 0.65 % Ca level; T5: 0.90 %, 0.75 %, 0.60 % Ca level, for starter, grower and finisher respectively. NPP levels were fixed to 2:1 ratio for each treatments.
[1]T test was applied during 0-10 days because of all treatment groups were received same Ca and NPP level compared to T1.
[a-c]Values within a column not sharing the same superscript are significantly different (P < 0.05). SEM: standard error of mean.

Table 3. Effects of different dietary Ca and NPP reduction at different periods on carcase parameters and tibia bone characteristics.

Treatments	Carcase parameters			Tibia bone characteristics		
	Carcase yield g/BW, %	Breast g/BW, %	Drumsticks g/BW, %	Tibia weight g/BW, %	Tibia ash % of tibia DM weight	Tibia phosphorus % of tibia ash weight
T1	68.11	32.06	20.11	0.44	36.59	17.36
T2	67.40	32.15	20.00	0.42	36.13	17.44
T3	67.41	31.48	20.07	0.42	36.04	17.33
T4	67.87	32.07	20.06	0.43	35.07	17.33
T5	68.03	31.60	20.24	0.44	35.74	17.31
SEM	0.444	0.415	0.226	0.011	0.526	0.101
P-linear	0.837	0.440	0.651	0.565	0.090	0.785
P-quadratic	0.182	0.937	0.549	0.129	0.462	0.801

T1: 0.96 %, 0.87 %, 0.78 % Ca level; T2: 0.90 %, 0.79 %, 0.65 % Ca level; T3: 0.90 %, 0.79 %, 0.60 % Ca level; T4: 0.90 %, 0.75 %, 0.65 % Ca level; T5: 0.90 %, 0.75 %, 0.60 % Ca level, for starter, grower and finisher respectively. NPP levels were fixed to 2:1 ratio for each treatments.

of dietary Ca and NPP levels through T2 to T5 treatments.

The results related to Ca and NPP intakes as absolute (g) and % of BW for the treatments were given in Table 4, and relationship between total Ca intake and BWG and FCR was shown in Figure 1. As shown in Table 4, dietary Ca and NPP reduction of T2 to T5 treatments during GP and FP at any studied levels resulted in significant linear and quadratic decline (P<0.001) in the total Ca and NPP consumption of broilers through 40 days feeding period, and when the relationship between total Ca intake and growth performance in Figure 1 considered, increasing total Ca intake significantly reduced the BWG and feed utilization (P<0.01).

Table 4. Effects of different dietary Ca and NPP reduction at different periods on Ca and NPP intakes.

Treatments	Total Ca intake (g)	Ca intake (% of BWG)	Total NPP intake (g)	NPP intake (% of BWG)
T1	35.06a	1.44a	17.53a	0.72a
T2	29.90b	1.22b	14.95b	0.61b
T3	28.32d	1.18c	14.16d	0.59c
T4	29.41c	1.21b	14.70c	0.60d
T5	28.18d	1.15c	14.09d	0.57e
SEM	0.121	0.008	0.061	0.004
P-linear	< 0.001	< 0.001	< 0.001	< 0.001
P-quadratic	< 0.001	< 0.001	< 0.001	< 0.001

T1: 0.96 %, 0.87 %, 0.78 % Ca level; T2: 0.90 %, 0.79 %, 0.65 % Ca level; T3: 0.90 %, 0.79 %, 0.60 % Ca level; T4: 0.90 %, 0.75 %, 0.65 % Ca level; T5: 0.90 %, 0.75 %, 0.60 % Ca level, for starter, grower and finisher respectively. NPP levels were fixed to 2:1 ratio for each treatments.

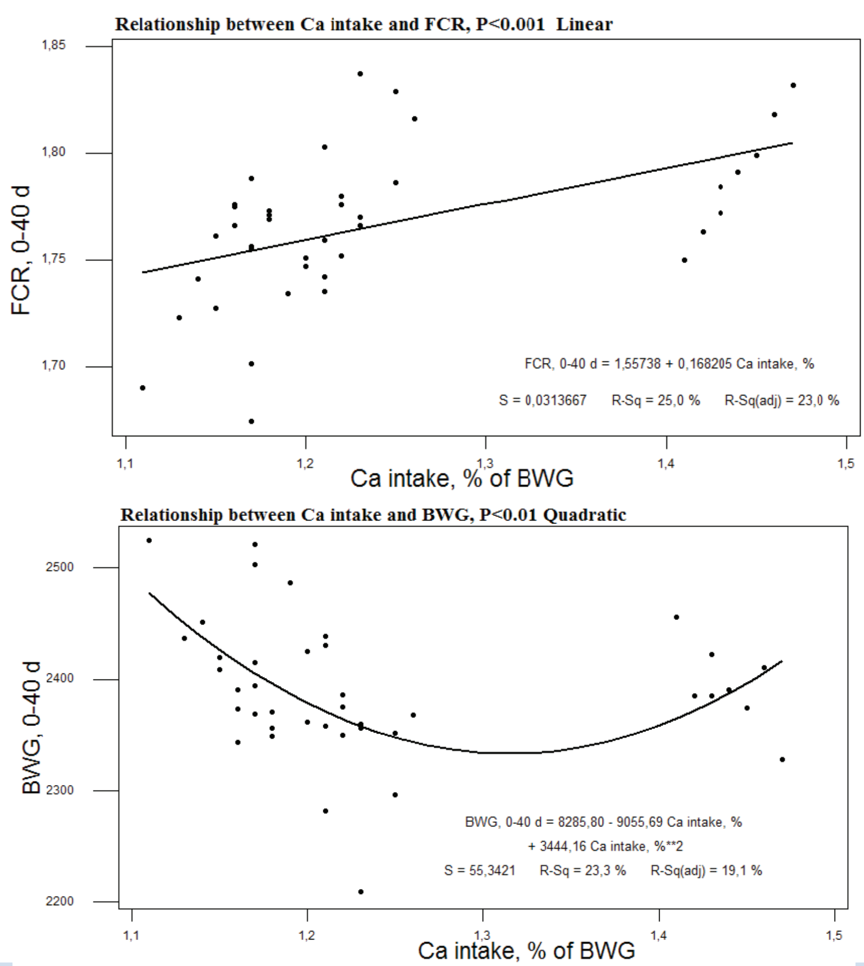

Figure 1: Relationship between Ca intake and body weight gain (BWG), and feed conversion ratio (FCR) of broilers

DISCUSSION

Although there is uncertainty regarding to which level dietary Ca and NPP can be reduced without compromising growth performance, it is important to consider that the bone development of broilers is dependent to age. Sanchez-Rodriguez et al. (2019) observed that during the earliest stages of growth (first 2 wks), the correct mineralization and structuring of bone is compromised. Besides Imari et al. (2020) reported that the adverse effects of 30% low dietary Ca with constant 2:1 ratio of NPP, from 0.96 to 0.67 %, was very clear on growth performance during the 0-10 d starter phase, but these effects were disappeared in the later grower and finisher periods. In line with our study, slight reduction at starter period, Valable et al. (2018) who kept the dietary Ca and P level steady during starter phase, and reduced the levels at grower and finisher periods. In contrary to the above mentioned strategy, some other researchers had successful growth performance comparable to control chicks with reduced Ca level (Létourneau-Montminy et al. 2010; Hamdi et al., 2015; Li et al., 2020; Kiani and Tahiri, 2020), even down to 0.61 % Ca (Kop-Bozbay et al., 2020) in the experiments lasted 2 or 3 wks of age. But they also reported that tibia weight and bone mineralization were significantly influenced by the level of Ca, with the low-calcium diet showing the lowest bone weight and ash content (Hamdi et al., 2015; Kop-Bozbay et al., 2020). This is a critical statement on the bone development which may subsequently induced the retarded growth rate in the remaining life span of broilers, and must kept in mind when Ca and NPP level are intended to reduce through entire feeding periods. Hence, one should consider that dietary Ca and NPP levels of starter diets needs to be safe enough, above 0.90 and 0.45%, respectively according to the present results, to achieve better growth rate and bone mineralization thereafter.

In our study, Ca and NPP reduction applied with by keeping a constant 2:1 ratio through entire feeding period of broilers did not influence growth performance, even T5, except FI. Besides, the regression analysis on Ca intake also demonstrated that high Ca intake could significantly reduce BWG (quadratic, $P<0.05$) and FCR (linear, $P<0.05$) (Figure 1). These results are in agreement with the findings of our previous report (Ceylan et al., 2020) where a similar approach with wider treatments applied. In line with our present study, Majeed et al. (2020) mentioned that Ca reduction from 0.90 % to 0.76 for first 16 days and from 0.76 to 0.58 for 17-35 days did not affect the performance results without phytase presence. Valable et al. (2018) also showed that, balanced to NPP, Ca reduction down to 0.60 % and 0.48 % at 11-21 and 22-37 days, respectively (without reduction during starter period) had no negative impacts on performance, but adversely affected tibia mineralization.

Our finding about negative relationship between higher Ca intake and BWG and FCR may be attributed to the deleterious effects of potential extra dietary Ca and its dominant source limestone for broiler chicks. As explained by many recent reports excess or imbalanced Ca to NPP may negatively influence the absorption and utilisation of P and micro minerals due to lower propensity for Ca-phosphate and Ca-phytate complexes in the digestive tract (Hamdi et al., 2015), and reduced energy availability by owing to formation of insoluble salts with dietary fatty acids (Delezie et al., 2012; Li et al., 2015) and to even decreased digestibility of protein and amino acids because of high acid-binding capacity of limestone which increase the pH and decrease the solubility of proteins (Selle et al., 2009; Walk et al., 2012). The less total Ca and NPP consumption (almost 20 % in T5) for per kg live body weight (linear and quadratic, $P<0.05$) in the treatment groups (T2 to T5) compared to the control were may also be an evident to above mentioned suggestions as no impaired growth performance obtained in the experiment. These successful results with the reducing strategy in the present experiment may also potentially be tied to the ability of poultry to utilize phytate P increases with age, result of more endogenous phytase, which is present in the gastrointestinal tract of older birds (Marounek et al., 2010).

Interestingly, reduced dietary Ca and NPP resulted in significantly low feed consumption in the treatment groups (quadratic, $P<0.05$), accompanied with better numerical FCR and similar BWG with the control diet. This is not in line with the previous reports indicating that no difference in FI was observed related to the reduced Ca and P at constant 2:1 ration in broilers (Yan et al., 2005; Venalainen et al., 2006; Mello et al., 2012). Although FI in poultry has been mainly related to dietary energy, the effect of dietary AMEn on FI has been questioned with modern broiler genetics (Classen, 2017). The response of growing broilers to energy density is variable, and may depend on several factors such as bird gender, breed, age, etc., including amino acid density, fat type and inclusion level as well as fiber type (Classen, 2017). In addition to the mentioned factors, Sharma et al. (2018) showed that dietary NPP level drives FI and interacts with dietary energy in broilers, resulting a significant decrease with low level of NPP (0.30 %) when AMEn increased, which is in line with the present results on FI. The reduction of FI in case of feeding NPP reduced diets could be the result of a loss of appetite (Letourneau-Montminy et al., 2010). However, there is very limited reports on the relationship between low level of NPP and FI, especially in the case that BWG and FCR were not influenced.

The high inclusion levels of inorganic P in poultry diets contribute to increased feed cost and environmental pollution due to excess P excretion (Delezie et al., 2015). Abudabos (2012) reported that the portion of P that exceeds the requirement will be excreted in faeces and P excretion is closely related to the P intake. As seen in Table 4, less Ca and NPP intake for each kg BWG up to 15 to 20 % in T2 to T5 diets, respectively compared to the control without compromising the growth performance and bone mineralization would not only save dietary cost, but also contribute to alleviate the environmental pollution e.g. eutrophication which may partly originated from high P content of broiler diets (Selle and Ravindran, 2007). Therefore, redefining Ca and NPP levels for broilers has become a major issue for the poultry industry, with economic and environmental aspects.

Similar to the growth performance, tibia ash, and P concentrations were not affected by the reduced level of dietary Ca and NPP, which indicates that the reduction down to 0.60 and 0.30 % in finisher periods may not have any negative effects on bone mineralization of growing broiler chickens. However, our result contradicts the assumption which indicates higher requirement of Ca and NPP is more necessary to improve bone mineralization than performance. It is expected that the ratio between the skeleton and soft tissues has been changed in contemporary broilers compared to broiler strains of one or two decades ago, which consequently affects the N, Ca and P content in broiler carcasses (van Krimpen et al., 2013). Onyango et al. (2003) found that bone-mineral content, bone mineral density, and percentage of ash increased linearly as the level of dietary Ca increased from 0.45 to 0.91%, which may be related to the much lower level of Ca than the applied level of the present study. However, some research agrees with the present study results, Rao et al. (2006) reported that reduced dietary Ca level down to 0.60 % from 0.90 % did not have any negative impact on bone mineralization of broilers. Diaz-Alonso et al. (2019) also showed that reduction of Ca level did not affect tibia ash and performance. Moreover, Kim et al. (2017) showed that with phytase presence, Ca reduction did not affect bone mineralization and bone strength. Therefore, it seems that modern broilers, with appropriate 2:1 ratio, might tolerate lower level of Ca and NPP than breeder's recommendations. The similar bone development even with 20% reduced T5 group to the control may be explained by the scenario, keeping Ca to NPP ratio constant at 2:1, and applying the reduction mainly post starter period.

CONCLUSION

The results of the present study demonstrated that by keeping these two minerals at a constant 2:1 ratio, lowering dietary Ca and NPP up to 20 % is possible through the grower and finisher phase without adversely affecting growth performance and tibia mineralization. Furthermore, regression analysis of Ca intake also indicated that recommended level Ca consumption may impair BWG and FCR. In conclusion, present study suggest that for Ross-308 broilers Ca and NPP requirements seem to be lower than the Aviagen recommendations in 2014 and 2019. Further, reducing these requirements especially after the starter period might help to both alleviate the environmental concern and save the dietary cost. However, further investigation need to be continued to support performance and tibia characteristic parameters by especially focussing on gut health, serum parameters, other nutrient digestibility studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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