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Effect of probiotic on growth performance and bone characteristics of broilers fed to a basal diets restricted by calcium and available phosphorus

تأثير المعزز الحيوي (Probiotic) في أداء النمو وخصائص العظام لفروج اللحم المغذاة على علائق قياسية مقيده بالكالسيوم و الفسفور المتاح

Zeyad Kamal imari , Ahmad Hassanabadi , Hassan Nassiri-Moghaddam

Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
hassanabadi@um.ac.ir

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Abstract

This study was conducted at the poultry farm to animal science department, Faculty of Agriculture, Ferdowsi University of Mashhad, for the period from 12/8/2018 to 26/9/2018. The objective of the study was to evaluate the influence of different levels of calcium (Ca) and available phosphorus (AP) with or without probiotic (Pro) on overall growth performance and criteria of the bones of broiler chickens. Four diets were formulated and fed to broilers from days 1 to 10 with different mineral status;1) standard diet (0.96% Ca and 0.48% AP) as recommended by the Ross 308 strain guidelines; 2) Low1 (0.864 % Ca and 0.432% AP); 3) Low2 (0.768 % Ca and 0.384% AP); and 4) Low3 (0.672 % Ca and 0.336% AP). On the eleventh day, each treatment of low-Ca and AP treatments except the standard treatment (control group) was divided into six groups (low1, low2, and low3 with Probiotic) or (low1, low2 and low3 without probiotic). The bacteria concentration was (2 x 10^9 CFU/g). While the restriction was continued until the end of trial. Results showed that lowering the Ca and AP levels did not influence on weight gain (WG) and feed intake (FI), but the feed conversion ratio (FCR) was influenced by reduced Ca and AP levels (P=0.036). Birds fed diet containing low-Ca and AP with probiotic or without it, had a better feed conversion ratio compared with control group (P=0.01 and P=0.005) respectively. The percentages of ash, calcium, and phosphorus of tibia were decreased in birds fed the low-Ca and AP diets with or without probiotics compared with the control group. Broiler chickens fed with the low-calcium and phosphorus+ probiotics diets showed a higher percentage of calcium and phosphorus in the tibia ash compared to those fed on the same diets without probiotics. The results also showed that the length, width and relative weight of femur were not influenced by Ca and AP-deficiency or by probiotic supplementation, but the breaking strength was influenced by calcium and available phosphorus deficiency. Whereas the breaking strength has reduced in birds that suffer Ca and AP deficiency with or without probiotic compared with the control group. In conclusion, it is possible to decrease dietary Ca and AP levels by 10% or 20% during the grower and finisher phases without affecting growth performance and bone criteria.

Keywords: calcium, available phosphorus, probiotic, growth performance, bone characteristics

المستخلص

اجريت الدراسة في حقل الطيور الداجنة التابع لقسم علم الحيوان، كليه الزراعة، جامعة فردوسي، مشهد للفترة من 12/أغسطس/ 2018 إلى 26/ سبتمبر/2018. كان الهدف من هذه الدراسة هو تقييم تأثير مستويات مختلفة من الكالسيوم (Ca) والفوسفور المتاح (AP) مع أو بدون المعزز الحيوى (Pro) فى أداء النمو الكلي ومعايير العظام لفروج اللحم. تم صياغة اربع علائق و غذيت بها الطيور من 1 الى 10 ايام و كانت العلائق كالاتي: 1) عليقة قياسية (0.96% كالسيوم و 0.48% فسفور متاح) حسب توصيات الدليل الارشادي لسلالة روس 308؛ 2) Low1 (0.864% كالسيوم و 0.432% فسفور متاح) ؛ 3) Low2 (0.768% كالسيوم و 0.384% فسفور متاح)؛ 4) Low3 (0.672% كالسيوم و 0.336% فسفور متاح). في اليوم الحادي عشر من العمر (مرحلة النمو) تم تقسيم كل معاملة من معاملات التي تحتوي على مستويات منخفضة من الكالسيوم و الفسفور ما عدا معاملة السيطرة (control group) الى ستة معاملات (low1, low2, and low3 with Probiotic) او (low1, low2, and low3 without Probiotic). حيث كان تركيز البكتريا (2×10^9 CFU/g). بينما استمر نقص الكالسيوم والفوسفور حتى نهاية التجربة. اظهرت النتائج ان انخفاض الكالسيوم و الفسفور لم يؤثر على الزياده الوزنية واستهلاك العلف، لكن كفاءة تحويل العلف قد تأثرت بخفض مستوى الكالسيوم و الفسفور(P=0.036) اذ امتلكت الطيور المغذاة على علائق تحتوي على مستويات منخفضة من الكالسيوم و الفسفور المتاح مع او بدون المعزز الحيوي، افضل كفاءة تحويل علف مقارنة مع معاملة السيطرة (P=0.01 and P=0.005) على التوالي. انخفضت نسبة الرماد، الكالسيوم و الفسفور في قصبة ساق الطيور المغذاة على علائق تحتوي على مستويات منخفضة من الكالسيوم و الفسفور مع او بدون المعزز الحيوي مقارنة مع مجموعة السيطرة. اظهرت الطيور التي تعاني من نقص الكالسيوم و الفسور و التي تم تغذيتها على عليقة تحتوي على المعزز الحيوي، نسبة اعلى من الكالسيوم والفسفور في قصبة الساق مقارنة مع الطيور التي تعاني من نقص الكالسيوم و الفسور و التي لم يتم تغذيتها على المعزز الحيوي. اظهرت النتائج ايضا ان طول، عرض و الوزن النسبي من عضمة الفخذ لم تتأثر بانخفاض الكالسيوم و الفسفور المتاح او باضافة المعزز الحيوي. لكن قوة الكسر لعضمة الفخذ قد تأثرت حيث انخفضت قوة الكسرفي الطيور التي تعاني من نقص الكالسيوم و الفسفور مع او بدون المعزز الحيوي مقارنة مع مجموعة السيطرة. في الختام، ان من الممكن خفض مستويات الكالسيوم و الفسفور الغذائية بنسبة 10% أو 20% خلال مرحلتي النمو و النهائية دون التأثير على أداء النمو و معايير العظام.

الكلمات الدالة: الكالسيوم، الفسفور المتاح، المعزز الحيوى، أداء النمو، صفات العظام.

Introduction

Corn, wheat, and soybean are considered as the main ingredients which are involved in formulation diets in the poultry nutrition, it is an important as a source of energy, protein, and fat. Besides, they are important sources of total phosphorus [1]. These ingredients contain over 60% of the total phosphorus, salts of phytic acid for instance (myo-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate), also known as phytate, which has been reported to be poorly digested by monogastric animals [1]. The phytate possess the ability of linking with other nutrients and digestive enzymes, resulting in lower nutrient digestibility and increased nutrient excretion in manure [2]. Subsequently, the amount of entered phosphorus to the environment by manure monogastric animals is very considerable, especially in areas with intensive livestock production. The phosphorus in poultry manure can cause environmental problems such as surface water eutrophication [3]. Increasing the level of calcium (Ca) in the diets, may reduce the digestibility of energy by chelating a portion of the available lipid fraction and subsequently, unavailability of some lipids for absorption [4]. Excess dietary Ca leads to the diminished availability of other minerals like P, Mg, Mn, Zn through the formation of Ca-phytate complexes and reducing the efficacy of phytase [5]. Lowering Ca and phosphorus (P) content in poultry diets can increase its utilization efficiency of Ca and P by improving digestive and absorption processes. Plumstead *et al.* [1] pointed out that the apparent digestibility and absorption of calcium, phosphorus, and Phytate-phosphorus were increased linearly when the levels of Ca dietary decrease from 1.16 to 0.47%. The phosphorus digestibility was significantly increased in birds that fed diet containing 0.6% Ca and 0.3% non-phytate phosphorus (NPP) compared with birds fed diets containing 1.0% Ca and 0.3% NPP or those the birds fed 1.0% Ca and 0.45% NPP [6]. The use of additives is one of important solutions to reduce the influence of P on the environment through reducing phytate-phosphorus excretion. Lin *et al.* [7] pointed out the using additives (enzymes, growth promoters, probiotics, prebiotics, vitamin D isomers, and organic acids) can improve the bioavailability of dietary nitrogen, P, and trace elements for animals. Probiotics are nonpathogenic bacteria which possess useful effects on the health or physiology of the host [8]. It is used to improve intestinal structure, developing immunity to defend against pathogens, and subsequently, improving growth performance [9, 10], meat quality [11], nutrient digestibility [12] and bone mineralization [13].

Materials and Methods

Birds and Management

The conditions and standards of rearing used in this experiment were approved by the Ferdowsi University of Mashhad Animal Ethic Committee. A total of 1440 one-day-old broiler chicks Ross-308 from a mixed flock (unsexed) was obtained from a local hatchery and randomly allocated to 120-floor pens (100 × 150 cm) covered with fine wood shavings as litter. On the first day, all birds were randomly allocated to four dietary treatments and the experimental design used in the starter phase was a completely randomized design (CRD). Each treatment included 36 replicates (pens) and 12 chicks per pen, except the control, contained 12 replicates and 12 chicks per pen. At day 11 of the experiment each treatment of low-Ca and AP treatments was divided into six treatments; each treatment contained six replicates (pens) and 12 chicks per pen, except the control treatment. Also, CRD was used in the grower and finisher phase. Each pen was provided with a suspended plastic chicken feeders and 4 nipple drinkers; both feeders and drinkers was adjusted according to the size of the birds. Birds received continuous artificial light during the first period (10 day) and then kept on 23L: 1D lighting schedule. The house temperature was initially maintained between 30- 32°C at the outset of the experiments, and then gradually reduced by 2°C every week to reach 21-23°C.

Table 1. Design of the experiment

Starter diet (1-10 d)[1]	Grower diet (11-24 d)[2]	finisher diet (25-42 d)[3]	treatments
Control (C)	Control (C)	Control (C)	CCC
	Low1	Low1	(L1L1L1)
Low1	Low2	Low2	(L1L2L2)
	Low3	Low3	(L1L3L3)
	Low1 + Probiotic	Low1 + Probiotic	(L1L1L1)+ Probiotic
	Low2 + Probiotic	Low2 + Probiotic	(L1L2L2) + Probiotic
Low2	Low3 + Probiotic	Low3 + Probiotic	(L1L3L3) + Probiotic
	Low1	Low1	(L2L1L1)
	Low2	Low2	(L2L2L2)
	Low3	Low3	(L2L3L3)
	Low1 + Probiotic	Low1 + Probiotic	(L2L1L1) + Probiotic
	Low2 + Probiotic	Low2 + Probiotic	(L2L2L2) + Probiotic

	Low3 + Probiotic	Low3 + Probiotic	(L2L3L3) + Probiotic
Low3	Low1	Low1	(L3L1L1)
	Low2	Low2	(L3L2L2)
	Low3	Low3	(L3L3L3)
	Low1 + Probiotic	Low1 + Probiotic	(L3L1L1) + Probiotic
	Low2 + Probiotic	Low2 + Probiotic	(L3L2L2) + Probiotic
	Low3 + Probiotic	Low3 + Probiotic	(L3L3L3) + Probiotic

[1]Control: 0.96% Ca, 0.48% AP; [1]L1: 0.864% Ca, 0.432% AP; [1]L2: 0.768% Ca, 0.384% AP; [1]L3: 0.672% Ca, 0.336% AP.
[2]Control: 0.87% Ca, 0.43% AP; [2]L1: 0.783% Ca, 0.387% AP; [2]L2: 0.696% Ca, 0.344% AP; [2]L3: 0.609% Ca, 0.301% AP.
[3]Control: 0.79% Ca, 0.395% AP; [3]L1: 0.711% Ca, 0.355% AP; [3]L2: 0.632% Ca, 0.316% AP; [3]L3: 0.553% Ca, 0.276% AP.

Dietary Treatments

Table (2) summarized treatment regimens. The nutritional program consisted of a starter diet for days 1-10, a grower diet for days 11 to 24, and a finisher diet for days 25- 42 of age based on the recommendations of the Ross 308 strain [14]. At day 1 to10, as presented in Table 1, the basal experimental diets were formulated to meet the broiler chickens nutritional requirements, according to Ross 308 strain with except calcium and available phosphorus levels. Dietary treatments included: a corn-soybean meal-based diet with recommended levels of Ca (0.96, 0.87 and 0.79%) and levels of AP (0.48, 0.435 and 0.395%) during starter, grower and finisher period, respectively as control treatment; L1 a corn-soybean meal-based diet with levels of Ca (0.864, 0.783 and 0.711) and levels of AP (0.432, 0.3915 and 0.3555) during starter, grower, and finisher period, respectively; L2 a corn-soybean meal-based diet with levels of Ca (0.768, 0.696, and 0.632) and levels of AP (0.384, 0.348, and 0.316) during starter, grower and finisher periods, respectively; and L3 a corn-soybean meal-based diet with levels of Ca (0.672, 0.609 and 0.553) and levels of AP (0.336, 0.3045, and 0.2765 %) during starter, grower and finisher periods, respectively, and two levels of probiotics were used in grower phase by (0 and 100 mg / kg diet) and in finisher phase by (0 and 50 mg / kg diet). Probiotic (Protexin) is a commercial probiotic which is manufactured by Probiotics International Ltd. England and was obtained from Nikotek Corporation-Tehran (Exclusive Agent in Iran). It is a multi-strain commercial preparation in powder form (2 x 10^9 CFU/g) that consists of *Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, Enterococcus faecium, Aspergillus oryzae*, and *Candida pintolopesii* [15, 16]. The manufacturer's recommended levels of Protexin supplementation are 0.01% (0.10 g/kg feed) until four weeks of age and 0.005% (0.05 g/kg feed) thereafter. All experimental diets were provided as mash form. All broilers had access to feed and water as ad libitum throughout the study (starter diet: 1 to 10, grower diet: 11 to 24, and finisher diet: 25 to 42 d of age).

Table 2. Composition and calculated analysis of experimental diets (as-fed basis).

Ingredient, g / 100g	Starter phase diet[1]				Grower phase diet[1]				Finisher phase diet[1]			
	Control	Low1	Low2	Low3	Control	Low1	Low2	Low3	Control	Low1	Low2	Low3
Corn	49.2	49.2	49.2	49.2	52.56	52.56	52.56	52.56	57.78	57.78	57.78	57.78
Soybean meal (44%)	41.56	41.56	41.56	41.56	37.79	37.79	37.79	37.79	32.29	32.29	32.29	32.29
Vegetable oil	4.53	4.53	4.53	4.53	5.41	5.41	5.41	5.41	5.97	5.97	5.97	5.97
Dicalcium phosphate[2]	1.93	1.65	1.37	1.08	1.71	1.46	1.2	0.94	1.54	1.31	1.07	0.84
Limestone[3]	1.06	0.97	0.88	0.79	0.98	0.9	0.82	0.74	0.91	0.84	0.76	0.69
Common salt	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
NaHCO3	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.38	0.38	0.38	0.38	0.32	0.32	0.32	0.32	0.29	0.29	0.29	0.29
L-Lysine HCl	0.25	0.25	0.25	0.25	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.18
L-Threonine	0.11	0.11	0.11	0.11	0.08	0.08	0.08	0.08	0.06	0.06	0.06	0.06
Choline	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Sand	0	0.37	0.74	1.11	0	0.33	0.67	1.01	0	0.31	0.61	0.92
Calculated composition[6]												
Metabolizable energy, kcal/kg	3000	3000	3000	3000	3100	3100	3100	3100	3200	3200	3200	3200
Crude protein, %	23	23	23	23	21.5	21.5	21.5	21.5	19.5	19.5	19.5	19.5
Crude fiber, %	3.99	3.99	3.99	3.99	3.8	3.8	3.8	3.8	3.53	3.53	3.53	3.53
Ca, %	0.96	0.864	0.768	0.672	0.87	0.783	0.696	0.609	0.79	0.711	0.632	0.553
Available P, %	0.48	0.432	0.384	0.336	0.43	0.387	0.344	0.301	0.395	0.355	0.316	0.276
Na, %	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Lysine, %	1.44	1.44	1.44	1.44	1.29	1.29	1.29	1.29	1.16	1.16	1.16	1.16
Methionine+ Cystine%	1.08	1.08	1.08	1.08	0.99	0.99	0.99	0.99	0.91	0.91	0.91	0.91
Threonine, %	0.97	0.97	0.97	0.97	0.88	0.88	0.88	0.88	0.78	0.78	0.78	0.78

Control= basal diet with 0.96, 0.87 and 0.79 % Ca and with 0.48, 0.43 and 0.39 % available Phosphorus during starter, grower and finisher phase, respectively; Low1= basal diet with 0.864, 0.783 and 0.711% Ca and with 0.432, 0.387 and 0.3555 % available Phosphorus during starter, grower and finisher phase, respectively; Low2= basal diet with 0.768, 0.696 and 0.632 % Ca and with 0.384, 0.344 and 0.316 % available Phosphorus during starter, grower and finisher phase, respectively; Low3= basal diet with 0.672, 0.609 and 0.553 % Ca and with 0.336, 0.301 and 0.2765 % available Phosphorus during starter, grower and finisher phase, respectively.

[1]Days 1 to10, days 11 to 24 and days 25 to 42

[2] 22% Ca and 17% P.

[3] 38% Ca. [4]The vitamin premix supplied the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 2,000 IU; vitamin E, 18 IU; vitamin K3, 2 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; vitamin B6, 3 mg; vitamin B12, 0.02 mg; Biotin, 0.15 mg; Pantothenic acid, 30 mg; niacin, 10 mg; choline chloride, 1,000 mg; vitamin C, 300 mg; and folic acid, 1 mg.

[5]The mineral premix supplied the following per kilogram of diet: Mn, 100 mg; Fe, 50 mg; Zn, 84.7 mg; Cu, 10 mg; I, 1 mg, and Se, 0.15 mg.

[6]Diets were formulated to meet nutritional requirements of broiler chickens according to Ross 308 strain nutritional recommendation (P and Ca content of the diets were adjusted by substituting washed sand).

Growth Performance

The body weight gain (BWG) and feed intake (FI) per pen were measured. Initial body weights (d 1) were subtracted from the final body weights to get BWG; feed consumption was calculated by subtracting residual feed from the offered feed. Data for feed consumption and BWG were used to calculate the feed conversion ratio (FCR). The FCR was adjusted for mortality and calculated on a per pen basis. The BWG, FI, and FCR were measured on days 10, 24, and 42 of ages. for calculating overall growth performance (1-42 day).

Laboratory Analyses

On 42 d, one bird (6 birds for each treatment) which had the closest body weight to the mean body weight for each treatment, was killed by cervical dislocation and the left tibias and right femurs were collected. The tibias and the femurs were cleaned from muscle and any adhering tissues using a scalpel and scissors. The length and width of femurs were measured using electronic caliper, before the right femurs breaking strengths test. The femurs were weighted using digital scale and afterward, bone breaking strength was measured on fresh femurs using an Instron testing machine (model H5K5, Tinius Olsen Company). The left tibias were defatted by soaking them in ethyl alcohol for 48 hours and then soaking them again in ethyl ether for 48 hours[17]. The left tibias were dried to a constant weight in a drying oven at 105°C for 12 hours, then they were changed to ash in a muffle furnace at 550°C for 12 hours [18]. The bone ash for each tibia was then digested with aqua regia and the calcium and phosphorus content of tibia ash were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES) [19].

Statistical Analysis

Statistical analysis of the data was conducted as a one-way ANOVA using GLM procedures [20] in a completely randomized design. Data were submitted to SAS (1996) for variance analysis and when significant, averages were compared by Tukey’s test ($P < 0.05$). Orthogonal contrast was also used to determine the mean comparison among treatments.

Results and Discussion

Overall growth performance (1-42 d)

Results revealed in table 3. Showed that the birds fed with low-Ca and AP diets had a similar average daily weight gain (ADWG) and average daily feed intake (ADFI) to those birds fed with standard diets. Moreover, there was a significant difference in feed conversion ratio (FCR) among treatments where the feed conversion ratio was significantly improved ($P<0.05$) in birds that fed diets containing low-Ca and AP without probiotic (L1L3L3, L3L3L3) or those that fed diet containing low-Ca and AP with probiotic (L1L2L2+pro) in comparison with the control group (Table 3). Also, in the orthogonal contrast (Control vs treatments without probiotic or Control vs treatments with probiotic) the feed conversion ratio (FCR) was significantly improved with low-Ca treatment with probiotics ($P=0.0150$) or without probiotics ($P=0.0059$) compared with control diet (Table 3). There were no significant differences for overall growth performance between low-Ca and AP treatments with probiotic and low-Ca and AP treatments without probiotic. This is in agreement with previous studies where Rousseau *et al.* [6] reported that the birds which fed on 0.6% Ca and 0.30% NPP diet had similar levels of growth performance to those fed on 1.0% Ca and 0.45% NPP diet, and pointed also the increased dietary Ca in low-NPP diets drastically impaired growth performance, mainly by reducing feed intake. Phosphorus levels can be reduced in the diet during the finisher stage without having any detrimental effects on bird performance [21]. The birds fed on 0.6% Ca, had maximum growth performance and nutrient retention when compared with birds receiving more than 0.6% Ca with level constant of NPP at 0.3% [22]. Rao *et al*. [23] reported that the birds fed on decreased levels of Ca from 1 to 42 d achieved higher growth performance at 28 and 42 d of age, especially for the lowest NPP levels (0.30 and 0.35%). Similarly, the birds fed on 0.60% Ca and 0.30% NPP achieved similar growth performance to control group [24].

Table3. Effect calcium and phosphorus restriction and probiotics supplementation on growth performance and tibia mineralization in broiler chickens

Dietary treatments[1]	Growth Performance 1-42 d			Tibia traits, 42 d		
	WG, g/b/d	FI, g/b/d	FCR, g/g	Ash%	Ca%	P%
CCC	56.78	88.00	1.54^{a}	50.57^{a}	36.93^{a}	17.86^{a}
L1L1L1	56.60	87.07	1.53^{ab}	49.59^{abc}	35.09^{bcde}	17.08^{ab}
L1L2L2	56.66	86.87	1.53^{abc}	49.51^{abc}	33.9^{cdef}	15.82^{defg}
L1L3L3	57.61	87.79	1.49^{c}	47.83^{bc}	32.66^{fg}	16.406^{bcde}
L2L1L1	58.99	88.42	1.52^{abc}	50.06^{ab}	35.73^{ab}	16.47^{bcde}
L2L2L2	56.88	86.07	1.52^{abc}	48.93^{abc}	35.32^{abcd}	15.42^{efgh}
L2L3L3	55.60	87.55	1.54^{a}	47.14^{cd}	30.79^{h}	14.64^{h}
L3L1L1	56.51	88.75	1.53^{ab}	47.43^{c}	35.51^{abcd}	16.99^{abc}
L3L2L2	56.03	84.92	1.51^{abc}	44.78^{d}	31.13^{gh}	14.82^{gh}
L3L3L3	54.93	82.30	1.50^{bc}	46.99^{dc}	31.29^{gh}	15.2^{fgh}
L1L1L1 + probiotic[2]	56.57	88.53	1.53^{ab}	49.27^{abc}	35.59^{abc}	17.06^{ab}
L1L2L2 + probiotic	58.27	87.99	1.51^{bc}	48.85^{abc}	34.84^{bcde}	16.35^{bcde}
L1L3L3 + probiotic	56.09	85.51	1.52^{abc}	47.48^{bc}	33.73^{def}	15.99^{cdef}
L2L1L1 + probiotic	56.59	87.14	1.53^{ab}	50.08^{ab}	35.32^{abcd}	16.98^{abc}
L2L2L2 + probiotic	55.03	84.29	1.53^{abc}	47.87^{bc}	35^{bcde}	16.25^{abcd}
L2L3L3 + probiotic	58.02	87.05	1.51^{abc}	47.86^{bc}	32.41^{fgh}	15.68^{defg}
L3L1L1 + probiotic	58.07	88.81	1.52^{abc}	49.37^{abc}	34.96^{bcde}	16.6^{bcd}
L3L2L2 + probiotic	57.70	86.10	1.52^{abc}	48.78^{abc}	33.46^{ef}	15.97^{cdef}
L3L3L3 + probiotic	56.61	87.19	1.54^{ab}	47.00^{cd}	31.13^{gh}	14.92^{gh}
SEM	0.236	0.356	0.0024	0.1776	0.1164	0.0742
P-values[3]	0.392	0.3010	0.0368	0.0001	<.0001	<.0001
			P-values			
CCC vs Low-Ca and AP[4]	0.8687	0.2680	0.0059	0.0024	<.0001	<.0001
CCC vs Low-Ca and AP+Pro[5]	0.8032	0.3998	0.0150	0.0128	<.0001	<.0001
Low-Ca and AP vs Low-Ca and AP+Pro	0.5086	0.6746	0.5732	0.1958	0.007	0.0329

BW= body weight; WG= weight gain; FI= feed intake; FCR= Feed conversion ratio.
[1]First letter corresponds to days 1 to 10; Second letter corresponds to days 11 to 24; third letter corresponds to days 25 to 42.
[2] Probiotic was used by 100 mg/kg of diet in the grower phase and 50 mg/kg of diet in the finisher phase.
[3,a,b,c,d] Values in the same column with different letters are significantly different ($P < 0.05$).
[4]Low calcium and available phosphorus treatments (Low-Ca and AP).
[5]Low calcium and available phosphorus + probiotics treatments (Low-Ca and AP+Pro).

Tibia traits

As shown in Table 3, the diet had an influence tibia ash percentage (P=0.0001), and also Ca (P<0.0001) and P (P<0.0001) percentages in tibia ash. The birds fed on the control diet had a higher level of studied traits above compared to those fed with the low-Ca and AP diets. Also, regarding to the orthogonal contrasts (control vs treatments without probiotic or control vs treatments with probiotic), the birds fed diets containing low-Ca AP with or without probiotics had a lower tibia ash and calcium and phosphorus percentage of tibia ash compared with those fed on control diet (Table 3). Supplementation of probiotic contributed to reduction effect of dietary Ca and P deficiency on bone health, where we observed that the birds fed diet with probiotic had a higher calcium and phosphorus percentage in the tibia ash compared with birds fed diet without probiotic. These results are in agreement with Onyango *et al*. [25] who found that bone-mineral content, bone mineral density, and ash percentage were increased linearly as the level of dietary Ca increased from 0.45 to 0.91%. The tibia ash and calcium percentage of tibia ash was increased by raising dietary P from 0.11% to 0.27% for the period of 1-21 days of age. Likewise, the phosphorus percentage of tibia ash was increased by increasing P dietary from 0.08% to 0.48% for a period of 22- to 42 days of age [26]. Mondal *et al*. [27] indicated that the tibia ash and calcium, phosphorus, and zinc percentages in tibia were decreased in the birds fed low-P diets (0.30% NPP) compared to the control diet (0.46% NPP). Similarly, the tibia ash was decreased by reducing dietary P level from 3.5 g/kg to 2.5 g/kg in the diet, while birds feeding on 10.0 g of Ca/ kg led to higher concentrations of tibia ash than for birds fed on 5.0 g of Ca/kg [18]. The slight decrease in ash, calcium, and phosphorus of tibia is probably due to the rise in PTH and 1,25$(OH)_2D_3$ hormone, which promotes the transfer of calcium from the bones to the blood. Dittmer and Thompson [28] how pointed out the 1,25$(OH)_2D_3$ together with PTH promotes mobilization of calcium from bones to maintain a constant blood calcium concentration. The 1,25$(OH)_2D_3$ hormone stimulates an increase of RANKL (receptor activator for NF-kB ligand), which in turn inducing osteoclastogenesis, resorption of bone, and mobilization of calcium [29].

Femur traits

Diets had no significant effects on length, width and relative weight of femurs measured on day 42, but the low Ca and P dietary had an influence on breaking strength of the femurs. As shown in Table 4, although the length and width of femurs at 42 days of age were not significantly influenced by diets, the results of orthogonal contrasts (control vs treatments without probiotic or control vs treatments with probiotics) showed that the length (P=0.0047) and width (P=0.0054) of the femurs were significantly increased in control treatment compared to low-Ca and AP treatments without probiotics (P=0.0094) or low-Ca and AP treatment with probiotics (P=0.0264). The birds fed on L3L3L3 diet and birds fed on L3L3L3+ pro diet achieved a lower average of breaking strength compared with all other treatments. It was also observed in orthogonal contrast that the breaking strength of femur was decreased in the birds fed diet containing low-Ca and AP with or without probiotic compared with control diet (P=0.016 and P=0.011, respectively).

This result is in agreement with the findings of Askari *et al*. [30] how showed that the length and width of femur and tibia were not influenced when Ca and P was reduced from 0.90% Ca and 0.45% P to 0.60% and 0.30%. The relative tibia weight was not influenced by reduced dietary Ca in the starter and finisher periods [31]. The length and width of tibia bone were not significantly influenced when Ca and P were reduced from 0.77% Ca and 0.32 % NPP to 0.37% Ca and 0.18% NPP, regardless of existence or absence of phytase

[32]. Deficiency of dietary Ca resulted in decline breaking strength, length, weight, ash weight and also calcium content in tibia ash [33]. Low dietary Ca decreased all mechanical strength parameters including stiffness (P=0.0002), modulus ($P < 0.0001$) and mixed force (P= 0.002) compared to control and high-Ca diets [34]. Breaking strength was dropped when dietary P level decreased from 0.45 to 0.30% in broilers fed with the 0.6% Ca diet, while the reduction was greater in those fed on 1.0% Ca diet [6]. Calcium to phosphorus ratio also has a significant effect on the health of the bones. Rao *et al*. [23] pointed out that the tibia breaking strength was decreased and leg abnormality score was increased in the birds fed on low levels of dietary P (3 and 3.5 g/kg NPP) with increasing Ca level in the diet at 14, 28 and 42 days of age. Chicks fed with the highest unbalanced diets (4.5 g NPP/kg and 5g Ca/kg) or (2.5 g NPP /kg and 9g Ca/kg), had the lowest tibia weight and tibia ash content [35].

Table 4. Effect calcium and phosphorus restriction and probiotics supplementation on broiler chickens femur criteria at 42 day

Dietary treatments[1]	Length (mm)	Width (mm)	Relative Weight %	Breaking Strength (N)
CCC	75.96	10.31	0.383	364.6^{a}
L1L1L1	71.92	9.455	0.365	320.0^{abc}
L1L2L2	74.33	9.242	0.366	290.5^{abcd}
L1L3L3	73.97	9.627	0.368	281.9^{bcd}
L2L1L1	74.23	9.751	0.381	312.9^{abc}
L2L2L2	73.33	9.61	0.369	335.1^{abc}
L2L3L3	73.43	9.595	0.353	300.3^{abc}
L3L1L1	73.75	9.787	0.363	349.5^{ab}
L3L2L2	72.39	9.667	0.344	286.5^{abcd}
L3L3L3	73.69	9.745	0.356	218.1^{d}
L1L1L1 + probiotic[2]	74.68	9.667	0.354	338^{abc}
L1L2L2 + probiotic	73.73	9.905	0.375	306.2^{abc}
L1L3L3 + probiotic	73.29	9.54	0.352	287.7^{abcd}
L2L1L1 + probiotic	74.6	10.225	0.377	332.1^{abc}
L2L2L2 + probiotic	73.78	10.03	0.382	321.1^{abc}
L2L3L3 + probiotic	73.51	9.71	0.358	308.2^{abc}
L3L1L1 + probiotic	73.9	9.912	0.358	289^{abcd}
L3L2L2 + probiotic	71.08	9.292	0.361	283^{bcd}
L3L3L3 + probiotic	74.32	9.535	0.35	265.7^{cd}
SEM	0.1911	0.0491	0.0029	5.442
P-values[3]	0.099	0.077	0.6921	0.021
		P-values		
CCC vs Low-Ca and AP[4]	0.0047	0.0054	0.1796	0.011
CCC vs Low-Ca and AP+Pro[5]	0.0094	0.0264	0.1805	0.016
Low-Ca and AP vs Low-Ca and AP+Pro	0.6008	0.1417	0.9944	0.7197

[1]First letter corresponds to days 1 to 10; Second letter corresponds to days 11 to 24; third letter corresponds to days 25 to 42.

[2] Probiotic was used by 100 mg/kg of diet in the grower phase and 50 mg/kg of diet in the finisher phase.

[3,a,b,c,d] Values in the same column with different letters are significantly different ($P < 0.05$).

[4]Low calcium and available phosphorus treatments (Low-Ca and AP).

[5]Low calcium and available phosphorus + probiotics treatments (Low-Ca and AP+Pro).

Conclusion

This study suggested that it is possible to decrease dietary calcium and available phosphorus levels by 10% or 20% of the Ross 308 recommendations during the grower and finisher phases without affecting growth performance because modern chickens are able to adapt to changes in dietary P and Ca through improvement of digestive efficiency.

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