Effect Different Levels of Calcium and Phosphorus With and Without Probiotics Inclusion on Growth Performance, Carcass Traits and Jejunum Morphology of Broiler Chickens

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Abstract

This study was conducted at the poultry farm of the Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, for the period from 12/8/2018 to 26/9/2018. The object of this study was to evaluate the impact of the inclusion of probiotics (Pro) on growth performance, carcass traits, and jejunum morphology in broiler chickens that suffering from calcium (Ca) and available phosphorus (avP) deficiency. A total of 1440 1-d-old broilers chicks (Ross 308) were used, on the first day, birds were randomly allotted to one of the 4 treatments: (1) control diet (0.96% Ca and 0.48% avP) as recommended by the Ross 308 strain guidelines; (2) Low1 (0.864 % Ca and 0.432% avP); (3) Low2 (0.768 % Ca and 0.384% avP); and (4) Low3 (0.672 % Ca and 0.336% avP). Each treatment contained on 36 replicates and 12 chicks for each replicate except the control treatment as it contained on 12 replicates and 12 chicks for replicate. On 11 d, each treatment of low Ca and avP treatments, except control treatment, were divided into the following six groups (low1, low2 and low3 with probiotics) or (low1, low2 and low3 without probiotics). The completely randomized design was used in the experimental design. The results showed no significant differences among treatments in terms of weight gain and feed intake, while the feed conversion ratio was impacted by low-calcium and phosphorus with or without probiotics. Where the feed conversion ratio has improved in the birds fed the Low Ca and avP diets or birds fed the Low-Ca avP+Pro diets when compared with those fed the control group ($P<.0001$). Dietary treatments did not affect the relative weight of parts and internal organs of the carcass ($P>0.05$). Additionally, the villus height, crypt depth and villus height to crypt depth ratio were improved in birds that fed low-calcium and phosphorus diets with Probiotics when compared with birds that fed low-calcium and phosphorus diets without probiotics. In conclusion, it is possible to decrease dietary Ca and avP levels by 10%, 20%, and 30% during the grower and finisher phases without affecting growth performance, carcass characteristics, internal organs, and intestinal morphology. Also, the addition of probiotics did not affect the traits studied above except for morphology intestinal.

Research paper from the Ph.D. thesis for the first author

Keywords: calcium, available phosphorus, probiotic, restriction, jejunum morphology

Introduction

Poultry produces about 20% of total fertilizer in animal farming but excretes about 36% of total phosphorus production [1]. The phosphorus excretion in animal manure can cause environmental problems such as surface water eutrophication [2]. The phosphorus excretion encourages the growth of blue-green algae, Overgrowth of certain blue-green algae is a concern because they produce toxins that are potential health hazards for animals and humans [3]. On the other hand, inorganic phosphorus is the third most expensive component in non-ruminant diets after energy and protein [4]. This element is a nonrenewable natural resource and its conservation is a global concern [5]. Therefore, the use of nutritional strategies is considered one of the important processes to reduce phosphorus excretion and improve use it. One of these strategies is applying early dietary P and Ca restriction which improves the efficiency of the animal to use dietary P [6]. Letourneau-Montminy *et al*. [7] Noted that the birds fed a low non-phytate P (NPP) and calcium (Ca) diet from 5 to 15 d of age (depletion period) exhibited equivalent growth performance and bone characteristics in the late period (repletion period). Powell *et al*. [8] Suggested that broilers fed lower levels of non-phytate phosphorus (nPP) in the starter phase are better able to adapt and grow at a low level of nPP in the growing phase than those fed a higher level of nPP in the starter phase. Another dietary strategy is the use of nutritional additives, probiotics one of the nutritional additives important are considered in the poultry industry. Where probiotic lactobacilli and other species of the endogenous digestive microflora are considered as an important source of the enzyme phytase which catalyses the release of phosphate from phytate [9]. Probiotics could improve growth performance, nutrient digestibility, humoral immunity, meat quality, increase gastrointestinal lactobacilli counts, decrease coliforms numbers and the manure ammonia emission in broilers [10]. The aim of this study was to investigate the effect of early dietary restriction of calcium in the starter phase (1-10) on a later phase at 42 days of age and study effect of inclusion of probiotics in broiler chickens diets that suffer calcium and phosphorus deficiency on growth performance, carcass parameters, and intestinal morphology.

Materials and Methods

Bird Husbandry

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 was included 36 replicates (pens) and 12 chicks per pen, except the control, was contained 12 replicates and 12 chicks per pen. At day 11 of the experiment each treatment of low-Ca and avP treatments was divided into six treatments, each treatment was 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 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, 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	Low1	(L1L1L1)
	Low2	Low2	(L1L2L2)
	Low3	Low3	(L1L3L3)
	Low1 + Probiotic	Low1 + Probiotic	(L1L1L1)+ Probiotic
	Low2 + Probiotic	Low2 + Probiotic	(L1L2L2) + Probiotic
	Low3 + Probiotic	Low3 + Probiotic	(L1L3L3) + Probiotic
Low2	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% avP; [1]L1: 0.864% Ca, 0.432% avP; [1]L2: 0.768% Ca, 0.384% avP; [1]L3: 0.672% Ca, 0.336% avP.
[2]Control: 0.87% Ca, 0.43% avP;[2]L1: 0.783% Ca, 0.387% avP; [2]L2: 0.696% Ca, 0.344% avP; [2]L3: 0.609% Ca, 0.301% avP.
[3]Control: 0.79% Ca, 0.395% avP;[3]L1: 0.711% Ca, 0.355% avP; [3]L2: 0.632% Ca, 0.316% avP; [3]L3: 0.553% Ca, 0.276% avP.

Dietary Treatments

The nutritional program consists of a starter diet of 1-10 days, a grower diet of 11 to 24 days, and a finisher diet of 25- 42 days of age based on the recommendation of the Ross 308 strain. From 1 to10 days, as shown in Table2, the basal experimental diets were formulated to meet the broiler chickens nutritional requirements recommended by Ross 308 strain with the exception of Ca and available phosphorus (AVP) levels. Calculated and analyzed nutritional compositions of all experimental diets are given in Table2. Dietary treatments were included: a corn-soybean meal-based diet with recommended levels of Ca (0.96, 0.87 and 0.79%) and levels of available Phosphorus (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 available Phosphorus (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 available Phosphorus (0.384, 0.348 and 0.316) during starter, grower and finisher period respectively; and L3 a corn-soybean meal-based diet with levels of Ca (0.672, 0.609 and 0.553) and levels of available Phosphorus (0.336, 0.3045 and 0.2765 %) during starter, grower and finisher period respectively. Two levels of probiotics were used in grower phase by (0 and 100 mg / kg) and in finisher phase by (0 and 50 mg / kg). 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 109 CFU/g) that consists of Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacteriumbifidum, Streptococcus thermophilus, Enterococcus faecium, Aspergillusoryzae and Candida pintolopesii. 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) from four weeks to the end trial. 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).

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 during 1-10 and 11-24 d of ages for calculating overall growth performance during 1-24 day.

Sample Collection

Feed was removed from all pens 4 h before slaughter. On d 42, 6 broilers per treatment (one broiler/pen) were randomly selected, weighed, slaughter and dissected individually, and sampled. Boneless and skinless breast, leg quarter, neck, back, wing, Heart, liver, gizzard, bursa, proventriculus, pancreas, spleen and Abdominal Fat were collected, weighed and recorded. A 1 cm of the midpoint of jejunum was also collected on day 42 then the jejunum samples were washed in phosphate-buffered saline (PBS) and immediately fixed in 10% buffered formalin for 72 hours histological examination [11].

Histomorphological Measurements

For morphometric analysis, intestinal samples were dehydrated and embedded in paraffin using routine methods. Consecutive sections (5 μm) were stained with hematoxylin and eosin before histomorphological evaluation on an Olympus BX 51 Microscope (Olympus Optical, pooyan servant medicine company, Iran [12]. The villus height and crypt depth were measured in at least 15 randomly selected villi and associated crypts per broiler at 10 × combined magnification [12].

Statistical Analysis

The statistical analyses for the experiment were performed using the GLM procedure by SAS Institute (Cary, NC). The experimental unit was the pen mean. Comparisons of the treatment means were performed with Duncan's multiple range test and orthogonal contrasts.

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).

Result and Discussion

Growth performance

The growth performance of birds feed different calcium, phosphorus and probiotics levels are shown in Table 3. No significant difference in average daily weight gain (ADWG) and average daily feed intake (ADFI) was observed among treatments. However, the feed conversion ratio was improved in birds fed L2L2L2 followed by L3L2L2+ Pro and followed some of low-calcium and phosphorus treatments compared to standard group (P=0.0065). Also, the results of orthogonal contrasts (control vs Low-Ca and avP or control Low-Ca and avP+Pro) showed that the ADWG and ADFI were influenced by neither calcium and phosphorus levels nor probiotics supplementation. In addition, birds fed diet with low-Ca and avP with and without probiotics have a better feed conversation ratio compared with control group. These finds were agreement with previous studies, L´etourneau-Montminy *et al*. [13] Reported that the birds fed diet with 0.48% Ca and 0.24% NPP have similar growth performance with birds fed diet with 0.70% Ca and 0.35% NPP at 35 day of age. The body weight, and feed intake were no influenced by reduced calcium dietary from 0.9% to 0.32% in the diets [14]. Birds which fed on 0.6% Ca and 0.30% nPP diet had similar growth performance to those fed on 1.0% Ca and 0.45% nPP diet [6]. Similarly, Kim *et al*. [15] exhibited that using different levels of calcium by (4, 5, 6, 7, 8 and 9 g/kg) have not impacted on body weight, body weight gain, feed intake, feed conversion ratio, and mortality. This result disagreed with Hamdi *et al*. [16] who pointed out that the increase of NPP in the diet from 3.0 to 4.0 g/ kg leads to an increased weight gain and feed intake between d 1 and d 21. Similarly, Han *et al*. [17] found that the feed intake and body weight gain were increased with increased dietary NPP levels form 0.25 % to 0.45% in broiler chickens from 1-21 d of age. Additionally, Li *et al*. [18] pointed out that the feed intake and weight gain decrease when the phosphorus dietary decrease from 0.50% to 0.27%, but the feed conversation ratio no influenced by phosphorus levels. The improvement in the productive performance of birds suffering from calcium and phosphorus deficiency may be due to the development of the adaptive mechanism. Bar *et al*. [19] pointed out that the intestinal calbindin synthesis and its mRNA were increased in the chickens fed the low-calcium or low-phosphorus diets from 19 to 26 days of age. Fang *et al*. [20] saw that the intestinal Na-Pi transporter activity has increased in birds fed the low-phosphorus (0.2%) diet compared with birds fed the normal-phosphorus (0.6%) or high-phosphorus (1.0%) diet. Centeno *et al*. [21] Demonstrated also that the feeding by a low levels of calcium enhances calcium uptake, Ca2+-ATPase, and Na+/Ca2+ exchanger activities and expressions. In addition, calcium pump (ATP2B1) mRNA increased 2 to 3 times in the duodenal plasma membrane which was reported in chickens fed either calcium or phosphorus deficient diet for 10 days [22].

Table 3. Effect calcium and phosphorus restriction and probiotics supplementation on growth performance of chicken broilers (1 to 24 day).

Dietary treatments[1]	Growth performance		
	WG, b/g/d	FI, b/g/d	FCR g/g
CC	34.19	50.97	1.49[a]
L1L1	34.74	49.41	1.42[bcd]
L1L2	34.07	48.59	1.42[bcd]
L1L3	34.74	50.56	1.42[bcd]
L2L1	35.46	50.78	1.43[bcd]
L2L2	34.99	49.06	1.40[d]
L2L3	34.76	51.05	1.47[ab]
L3L1	34.95	50.23	1.43[bcd]
L3L2	33.62	47.35	1.42[bcd]
L3L3	32.09	46.94	1.46[abcd]
L1L1+ Probiotic[2]	35.45	51.29	1.44[abcd]
L1L2+ Probiotic	35.81	50.06	1.41[bcd]
L1L3+ Probiotic	33.27	48.17	1.45[abcd]
L2L1+ Probiotic	35.18	50.81	1.44[abcd]
L2L2+ Probiotic	33.89	48.94	1.44[abcd]
L2L3+ Probiotic	34.54	49.36	1.43[bcd]
L3L1+ Probiotic	35.50	51.01	1.43[bcd]
L3L2+ Probiotic	33.99	48.15	1.42[cd]
L3L3+ Probiotic	33.71	49.14	1.45[abcd]
SEM	0.217	0.249	0.003
P-values[3]	0.5802	0.112	0.0065
P-values			
CCC vs Low-Ca and avP[4]	0.8057	0.0626	<.0001
CCC vs Low-Ca and avP+Pro[5]	0.5924	0.1347	<.0001
Low-Ca and avP vs Low-Ca and avP+Pro	0.6317	0.5334	0.6154

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

Carcass Traits

The results revealed that the birds fed diet containing calcium and phosphorus deficiency regardless of presence or absence of probiotics were possessed a similar relative weight of the breast, leg, back, wing, and abdominal fat compared with a control group (table 4). Also, in table 4. That the orthogonal contrasts exhibited that the relative weight of the breast meat, leg, back, wing, and abdominal fat no influenced by low calcium and phosphorus or probiotic supplementation. The results obtained are an agreement with previous studies reviewed by authors. Han et al. [23] Pointed out that the carcass, breast, and leg quarter relative weight were not influenced by non-phytate phosphorus levels. Reducing calcium and phosphorus levels did not impact on relative weight of carcass, breast, thigh+ drumstick, wing, head, feet, back, and abdominal fat [24]. The weight of the breast, thigh, drumstick, and wing were influenced by neither calcium levels nor phosphorus levels. Additionally, the results obtained were agreed with the findings by Pourakbari et al. [25] who reported that the probiotics levels were no effect on the relative weight of the breast, drumstick, wing, abdominal fat, and carcass weight. Likewise, Dietary supplementation by 0.1%, 0.5%, or 1% of multistrain probiotic did not affect (p>0.05) on the relative weight of the breast, drumstick, wing, and abdominal fat [26]. Whereas the results obtained do not agree with the findings by Salehizadeh et al. [27] who indicated that the addition of probiotics in its kinds of the local and imported has increased the relative weight of carcass, breast, drumstick and has decreased abdominal fat. Similarly, Atela et al. [28] Confirmed that the birds fed a diet with probiotics have great breast, wing, thigh, and shank weights compared to the control group. Some of the authors have observed that inclusion probiotic to diets reduces abdominal fat weight in broilers compared with the controls [29], [30].

Table 4. Effect calcium and phosphorus restriction and probiotics inclusion on broiler carcass criteria at 42 day.

Dietary treatments[1]	Carcass traits%				
	Breast	Leg quarter	Neck + Back	Wing	Abdominal Fat
CCC	25.56	19.67	15.74	5.32	1.35
L1L1L1	26.43	19.75	15.32	5.45	1.53
L1L2L2	26.10	19.96	14.75	5.16	1.32
L1L3L3	27.11	19.70	15.20	5.29	1.27
L2L1L1	27.02	20.23	15.20	5.44	1.39
L2L2L2	26.86	19.26	16.02	5.27	1.40
L2L3L3	26.56	20.36	14.95	5.27	1.55
L3L1L1	27.47	19.50	15.12	5.27	1.11
L3L2L2	27.53	19.97	15.77	5.24	1.48
L3L3L3	25.90	19.85	15.89	5.39	1.61
L1L1L1+ Probiotic[2]	27.06	20.55	15.46	5.29	1.86
L1L2L2+ Probiotic	27.41	20.14	15.87	5.22	1.24
L1L3L3+ Probiotic	26.59	19.96	15.51	5.22	1.35
L2L1L1+ Probiotic	25.95	19.54	15.09	5.16	1.49
L2L2L2+ Probiotic	24.86	19.02	15.17	5.05	1.31
L2L3L3+ Probiotic	26.11	19.04	14.83	5.08	1.14
L3L1L1+ Probiotic	25.43	19.18	15.23	5.18	1.05
L3L2L2+ Probiotic	24.98	19.62	15.29	5.32	1.18
L3L3L3+ Probiotic	25.59	19.310	14.55	5.42	1.56
SEM	0.1967	0.0835	0.0923	0.0307	0.0398
P-values	0.5687	0.1198	0.336	0.7802	0.210
	P-values				
Control vs Low-Ca and avP[3]	0.1787	0.6511	0.3465	0.9663	0.7686
Control vs Low-Ca and avP+Pro[4]	0.6267	0.8342	0.2022	0.4739	0.993
Low-Ca and avP vs Low-Ca and avP+Pro	0.059	0.1536	0.4560	0.1409	0.4994

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

Relative weight of internal organs of carcass

No differences were observed among the treatments in terms of the relative weight of the heart, liver, gizzard, pancreas bursa, spleen and proventiculus (Table 5). Also, the results of orthogonal contrasts showed no significant difference between the control group and the Low-Ca and avP groups or between control groups and the Low-Ca avP+Pro groups or between the Low-Ca and avP groups and the Low-Ca avP+Pro groups. This finding agrees with data published by de Freitas *et al*. [24] who reported that the relative weight of proventriculus, gizzard, pancreas, small intestine, and heart were no influenced by reduced calcium and phosphorus levels. Han *et al*. [23] who also found the relative weight of carcass organs was not influenced by non-phytate phosphorus levels. Likewise, Akter *et al*. [31] Pointed out the relative weight of organs was no impacted when reducing calcium and phosphorus from (10 g/kg Ca and 4 g/kg nPP) to (6 g/kg Ca and 3 g/kg nPP). The result obtained was agreed with finds Salehizadeh *et al*. [27] who pointed out the relative weight of the liver, gizzard, and heart was not impacted by probiotics supplementation. Likewise, no significant differences in the relative weight of liver, bile, spleen, thymus, and bursa between the control group and a probiotic group [25], [26], [28].

Table 5. Effect calcium and phosphorus restriction and probiotics inclusion on relative weight of carcass organs at 42 days.

	Relative weight of internal organs %						
Dietary treatments[1]	Heart	Liver	Gizzard	Pancreas	Bursa	Spleen	Proventriculus
CCC	0.413	1.85	1.49	0.247	0.123	0.108	0.325
L1L1L1	0.418	1.72	1.50	0.236	0.152	0.103	0.320
L1L2L2	0.455	1.89	1.37	0.236	0.144	0.095	0.337
L1L3L3	0.398	1.75	1.39	0.226	0.122	0.119	0.313
L2L1L1	0.414	1.78	1.49	0.232	0.141	0.114	0.287
L2L2L2	0.430	1.81	1.61	0.255	0.191	0.112	0.368
L2L3L3	0.429	1.82	1.50	0.238	0.127	0.116	0.318
L3L1L1	0.428	1.71	1.44	0.215	0.141	0.116	0.313
L3L2L2	0.455	1.72	1.35	0.225	0.126	0.099	0.340
L3L3L3	0.414	1.77	1.43	0.213	0.177	0.113	0.301
L1L1L1+ Probiotic[2]	0.455	1.67	1.37	0.212	0.128	0.114	0.305
L1L2L2+ Probiotic	0.465	1.61	1.44	0.226	0.108	0.096	0.319
L1L3L3+ Probiotic	0.484	1.87	1.46	0.239	0.144	0.119	0.318
L2L1L1+ Probiotic	0.418	1.88	1.30	0.210	0.119	0.113	0.268
L2L2L2+ Probiotic	0.400	1.95	1.56	0.215	0.117	0.105	0.315
L2L3L3+ Probiotic	0.393	1.83	1.37	0.236	0.128	0.110	0.332
L3L1L1+ Probiotic	0.426	1.88	1.31	0.244	0.155	0.130	0.346
L3L2L2+ Probiotic	0.427	1.79	1.48	0.216	0.111	0.111	0.341
L3L3L3+ Probiotic	0.440	1.74	1.48	0.221	0.157	0.115	0.342
SEM	0.0066	0.0205	0.0160	0.0036	0.0043	0.0030	0.0048
P-values	0.7925	0.5334	0.1779	0.8267	0.1973	0.9829	0.2828
P-values							
Control vs Low-Ca and avP[3]	0.6865	0.4078	0.5913	0.5760	0.2317	0.8967	0.9069
Control vs Low-Ca and avP+Pro[4]	0.5290	0.600	0.3160	0.1921	0.7250	0.7292	0.8660
Low-Ca and avP vs Low-Ca and avP+Pro	0.5788	0.600	0.2966	0.0957	0.0604	0.6423	0.8987

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

Intestinal morphometric parameters

The results of the jejunum morphology of broilers in broiler chickens at day 42 are shown in Table 6. The villus height, crypt depth, and villi width were influenced by the interaction of calcium and phosphorus levels and probiotics supplementation. The birds fed the L2L3L3+ Pro diet was recorded a great average of villus height followed by L3L3L3+

Pro diet compared with control diet. However, the Crypt depth was decreased in the L2L3L3+Pro and L3L1L1+Pro groups compared to control group. Also birds fed the L3L1L1 diet followed by L1L2L2+Pro diet have a higher mean of villus width compared to control diet. While the villus height to crypt depth ratio no influenced by the interaction of calcium and phosphorus levels and probiotics supplementation. The results of orthogonal contrasts (Control vs Low-Ca and avP+PRO) showed that the addition of probiotics to Low-calcium and phosphorus diets significantly increased villus height and villus height to crypt depth ratio when compared with the control group. Also, the villus width has increased in the Low-calcium and phosphorus groups compared to the control group (Control vs Low-Ca and avP). in addition, the villus height and villus height to crypt depth ratio have increased and the crypt depth has decreased in the Low-Ca and avP+PRO groups when compared with the Low-Ca and avP groups, These results are in agreement with the previous study which pointed out that the addition of *Bacillus subtilis* by 0.30 and 0.45% in the diet increased villus height and villus height to crypt depth ratio compared with the control diet [32]. Similarly, Awad *et al.* [33] Reported that inclusion of probiotics lead to increased villus height and villus height to crypt depth ratio in duodenum and decreased ileal crypt depth. Also, Lei *et al.* [34] pointed out that the villus height and villus height to crypt depth ratio of duodenum, jejunum, and ileum were significantly increased for birds fed with probiotics by 60 mg/kg compared to the control and virginiamycin groups ($p<0.05$). Whereas, Wang *et al.* [35] did not find any significantly different in jejunum morphology that included (villus height, villus width, crypt depth, and muscle thickness) among the probiotics groups and the control group. The results of orthogonal contrasts (Control vs Low-Ca and avP) in the current study revealed that the levels of calcium and phosphorus did not affect Jejunum morphology that included (villus height, crypt depth, and villus height to crypt depth ratio). These results were in agreement with the findings of Oikeh *et al.* [36] who pointed out that the birds fed diet containing deficient of calcium and phosphorus has a similar villus height, crypt depth, and villus height to crypt depth ratio in three sections of the small intestine (Duodenum, Jejunum, and Ileum), when compared with birds fed diet containing adequate of calcium and phosphorus.

Table 6. Effects of calcium and phosphorus deficiency and probiotic mixture on the morphology of jejunum of broilers at 42 day.

Dietary treatments[1]	Villus height (μm)	Crypt depth (μm)	VCR[2]	Villus width (μm)
CCC	1067.2[bc]	281.6[ab]	4.00	122.4[bc]
L1L1L1	1189.0[abc]	269.0[abc]	4.67	131.6[abc]
L1L2L2	1190.0[abc]	243.8[abcd]	5.00	137.4[abc]
L1L3L3	1149.6[abc]	272.8[abc]	4.35	142.8[ab]
L2L1L1	1034.4[bc]	289.2[a]	3.70	134.6[abc]
L2L2L2	1003.4[bc]	245.2[abcd]	4.14	136.0[abc]
L2L3L3	1038.9[bc]	254.6[abcd]	4.33	130.8[abc]
L3L1L1	1043.0[bc]	246.8[abcd]	4.48	147.6[a]
L3L2L2	1109.2[abc]	281.8[ab]	3.97	141.5[ab]
L3L3L3	1114.0[abc]	245.8[abcd]	4.69	138.0[abc]
L1L1L1+ probiotics[3]	1239.2[ab]	276.8[abc]	4.84	139.2[abc]
L1L2L2+ probiotics	1114.8[abc]	255.2[abcd]	4.63	147.4[a]
L1L3L3+ probiotics	1214.6[ab]	282.2[ab]	4.49	119.6[c]
L2L1L1+ probiotics	1149.4[abc]	240.0[abcd]	4.93	119.0[c]
L2L2L2+ probiotics	1167.6[abc]	253.4[abcd]	4.68	131.2[abc]
L2L3L3+ probiotics	1302.6[a]	225.4[cd]	5.40	129.0[abc]
L3L1L1+ probiotics	1204.6[abc]	215.0[d]	4.94	134.8[abc]
L3L2L2+ probiotics	1218.6[ab]	233.0[bcd]	5.25	136.8[abc]
L3L3L3+ probiotics	1301.6[a]	241.6[abcd]	5.51	130.7[abc]
SEM	13.914	3.514	0.086	1.405
P-values[4]	0.0127	0.0275	0.0734	0.0420
	P-values			
Control vs Low-Ca and avP[5]	0.6430	0.2059	0.3518	0.0180
Control vs Low-Ca and avP+Pro[6]	0.0251	0.0351	0.0163	0.1373
Low-Ca and avP vs Low-Ca and avP+Pro	0.0001	0.0555	0.0014	0.0481

[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] Villus height to Crypt depth Ratio
[3] Probiotic was used by 100 mg/kg of diet in the grower phase and 50 mg/kg of diet in the finisher phase.
[4,a,b,c,d] Values in the same column with different letters are significantly different ($P < 0.05$).
[5]Low calcium and available phosphorus treatments (Low-Ca and avP).
[6]Low calcium and available phosphorus + probiotics treatments (Low-Ca and avP+Pro).

Conclusion

This study indicates that reducing calcium and phosphorous by a moderate amount (10 and 20%), not only affects feed consumption and weight gain but also has improved from feed conversion rate. Also, decrease calcium and phosphorus levels did not impact on carcass traits, intestinal morphology and internal organs of broiler chickens. Additionally, that inclusion of probiotic improves villus height, crypt depth, and villus height to crypt depth ratio.

Conflict of Interests.
There are non-conflicts of interest .

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الخلاصة

اجريت الدراسة في حقل الطيور الداجنة التابع لقسم علم الحيوان، كلية الزراعة، جامعة فردوسي، مشهد للفترة من 12/أغسطس/ 2018 إلى 26/ سبتمبر/2018. كان الهدف من هذة الدراسة هو لتقييم تأثير ادراج المعزز الحيوي (Probiotics) في اداء النمو، خصائص الذبيحة و مورفولوجيا الامعاء لدى فروج اللحم التي تعاني من نقص الكالسيوم (Ca) و الفسفور المتاح (avP) . تم استخدام 1440 فرخا بعمر يوم واحد من سلالة (Ross)، في اليوم الاول تم تقسيم الطيور بشكل عشوائي الى اربع معاملات. (1) معاملة السيطرة اذ احتوت على (0.96% كالسيوم و 0.48% فسفور متاح) حسب توصيات الدليل الارشادي لسلالة (Ross)، (2) Low1 (0.864% كالسيوم و 0.432% فسفور متاح)، (3) Low2 (0.768% كالسيوم و 0.384% فسفور متاح) و (4) Low3 (0.672% كالسيوم و 0.336% فسفور متاح). احتوت كل معاملة على 36 مكررا و 12 فرخا لكل مكرر باستثناء معاملة السيطره اذ احتوت على 12 مكررا و 12 فرخا لكل مكرر. في اليوم الحادي عشر من العمر تم تقسيم كل معاملة من معاملات منخفضة الكالسيوم و الفسفور الى ستة مجاميع و كانت كالاتي(low1، low2 و low3 مع المعزز الحيوي) او (low1، low2 و low3 بدون المعزز الحيوي). تم استخدام التصميم العشوائي الكامل في التصميم التجريبي. اظهرت النتائج بعدم وجود اختلافات معنوية بين المعاملات بالنسبة للزيادة الوزنية و استهلاك العلف، بينما تاثرت كفاءة تحويل العلف بمستويات الكالسيوم و الفسفور مع او بدون المعزز الحيوي. اذ تحسنت نسبة تحويل العلف في الطيور التي تغذت على علائق منخفضة الكالسيوم و الفسفور المتاح او الطيور المغذاة على علائق منخفضة الكالسيوم و الفسفور المتاح + المعزز الحيوي عند مقارنتها مع تلك التي تغذيت على عليقة السيطرة ($P<.0001$). كذلك لم تؤثر المعاملات الغذائية في الوزن النسبي للاجزاء و الاعضاء الداخلية للذبيحة ($P>0.05$). بالإضافة إلى ذلك، هناك تحسن واضح في ارتفاع الزغابة، عمق الكريبت و نسبة ارتفاع الزغابة الى عمق الكريبت في الطيور المغذاة على علائق منخفضة الكالسيوم والفسفور مع المعزز الحيوي عند مقارنتها مع الطيور المغذاة على علائق منخفضة الكالسيوم و الفسفور بدون المعزز الحيوي. في الختام، ان من الممكن خفض مستويات الكالسيوم و الفسفور الغذائية بنسبة 10%، 20% و 30% خلال مرحلتي النمو و النهائية دون التأثير على أداء النمو، خصائص الذبيحة، الاعضاء الداخلية و التشكيل المعوي. ايضا لم يؤثر اضافة المعزز الحيوي على الصفات المدروسة اعلاه باستثناء التشكيل المعوي.

البحث مستل من اطروحة الدكتوراه للباحث الاول

الكلمات الدالة: الكالسيوم، الفسفور المتاح، المعزز الحيوي، مورفولوجيا الصائم