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Research Article
Adaptability of Broilers to Dietary Phosphorus Repletion and Depletion Programs

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Abstract

Objective: This study was designed to evaluate the ability of broiler to adapt the diets with phosphorus (P) depletion and repletion. **Materials and Methods:** A total of160 broilers were housed in 20 pens. Two diets based on corn and soybean were formulated to pre-initial and initial phases: Control - formulated within nutritional recommendation; Reduced-without dicalcium phosphate -0.15% of available P. Treatments consisted of four P supply schemes: Normal P (NP)-control diet during entire trial; Depletion (DP)-Control diet in pre-initial and reduced level diet provided during the initial phase; Repletion (RP)-reduced diet during the pre-initial and control diet in initial phase and Reduced P (RL)-reduced diet during the pre-initial and initial phases. All broilers received control diet during grower phase. Body weight, feed intake and feed conversion were evaluated. Carcass yield and bone mineral contents were assessed at 21 and 28 days. Data were analyzed using ANOVA and Tukey test ($p<0.05$). **Results:** Broilers fed with RL diet showed impaired performance compared to NP treatment. Broilers fed with NP and RP diets presented higher weight gain and feed intake and similar tibia mineralization after 21 or 28 days. **Conclusion:** Although, practical application depends on further research, broilers fed phosphorus supplemented diet have the ability to recover body weight, weight gain and bone mineralization.

Key words: Bone mineralization, nutrient metabolism, poultry diet, growth performance, physiology, broiler

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Macrominerals exert important biological functions in the animal organism. Among them, phosphorus (P) has an important role in nutrient metabolism, nucleic acid synthesis, cell membranes, acid-base balance, cell differentiation and bone mineralization[1,2].

Dietary P is founded in cereal-based diets in the form of phytic P, which is not readily available for monogastric animals. Thus, the addition of high amounts of inorganic sources is necessary to meet the requirements, which makesP the most expensive mineral in feed formulation[3-5].

The adaptation of animals to low nutrient diets has been long recognized. In general, animals respond to nutrient restriction by increasing absorption rates and utilization efficiency, which decreases excretion of the restricted nutrients[6,7]. Previous studies in pigs have shown that a mineral restriction followed by a diet that meets or exceeds requirements improves mineral retention efficiency during reduction and subsequent repletion[8,9]. The higher efficiency of P retention due to reduction-repletion can rectify mineral deficiencies without affecting the performance of pigs[10]. To date, limited studies have been carried out to investigate the effects of this metabolic conditioning on performance and bone mineralization of broilers. Thus, this research was conducted to study the ability of broiler to adapt the feed programs with P depletion and repletion by evaluating performance, carcass yield and bone mineralization.

MATERIALS AND METHODS

This study was conducted at the Animal Science Laboratory (LEZO) of the Universidade Federal do Rio Grande do Sul from January to July 2019.All procedures were approved by the Animal Care and Welfare Committee of Federal University of Rio Grande do Sul under protocol 19551.

Bird husbandry: A total of 160 male Cobb 500 (45 g) broiler chickens were obtained directly from the hatchery and reared until 28 days. The broilers were housed in a building with 20 floor pens of 1 m^2 covered with wood shavings, equipped with nipple drinkers and feeders. On the first day of life, groups of 8 birds were weighed and distributed in pens and the variation in body weight among bird groups did not exceed 3%. The feed and water were provided *ad libitum* and environmental temperature was maintained at 32°C for the first week and was decreased 3°C each week thereafter.

Diets and treatments: Diets based on corn and soybean meal were formulated for three phases (pre-initial: 1-7, initial: 7-21 and growth: 21–28 days, Table 1). Control diets were formulated according to the nutritional recommendations of the Brazilian Tables for Poultry and Swine[11]. Two other diets (Reduced level) were used to compose the scheme of P depletion and repletion (Table 2). Treatments consisted of four phosphorus supply schemes: Normal phosphorus (NP), with control diet provided during the entire trial; Repletion phosphorus (RP), with reduced level provided only during the pre-initial phase; Depletion phosphorus (DP), with reduced level provided during only the initial phase and Reduced phosphorus (RL), with reduced level provided during pre-initial and initial phases. All broilers received control diet during grower phase. These feeds differed from the control in the absence of phosphate inclusion and phosphate was replaced by Celite. The level of available P was 0.47 (control) and 0.15% (reduction) in the initial phase and 0.40 (control) and 0.15% (reduction) in the grower phase. All animals received the control diet from 21-28 days, containing 0.34% of P in the diet. The main idea was the removal of di-calcium phosphate from the diet of broiler so there is no permanent association between phosphorus and calcium. Meanwhile, it was verified in the formulation that the diets showed no relation if it is less than 1:1.

Performance evaluation: Body weight (BW), feed intake (FI), body weight gain (BWG) and feed conversion rate (FCR) were evaluated weekly. The mortality rate was less than 3% and the weights of dead birds were used to adjust feed conversion ratios.

Carcass yield, tibia analysis and leg deformities: At 21 and 28 days, two birds per replicate were euthanized, weighed and the carcass yield was evaluated using the following formula:

$$\text{Carcass weight} = \frac{\text{Without head and viscera}}{\text{Weight of animal}} \times 100$$

The tibias of the slaughtered animals were removed and analyzed for dry matter (method 930.15) and ashes (method 923.03) according to AOAC[12]. All broilers that presented deformation in the legs were counted on the 28th day in order to evaluate the percentage of animals that presented leg deformation.

Statistical analysis: Birds were randomly assigned to 4 treatments (Normal phosphorus, Depletion, Repletion and

Table 1: Ingredients composition and nutritional levels of experimental diets fed to broilers receiving different levels of phosphorus from 1-28 days of age

	Pre-initial (1-7 days)		Initial (8-21 days)		Growth (22-28 days)
Items	Control	Reduced	Control	Reduced	Control
Ingredients (%)					
Corn	53.80	53.80	53.94	53.94	61.99
Soybean meal, 45% crude protein	39.33	39.33	38.53	38.53	30.47
Vegetal Oil	2.53	2.53	3.83	3.83	4.17
Dicalcium phosphate[1]	1.90	0.00	1.56	0.00	1.25
Celite	0.00	1.90	0.00	1.56	0.00
Limestone	1.00	1.00	1.00	1.00	0.88
Salt	0.51	0.51	0.48	0.48	0.46
L-Lys HCl	0.27	0.27	0.16	0.16	0.25
DL-met	0.36	0.36	0.29	0.29	0.28
L-Tre	0.10	0.10	0.04	0.04	0.07
Mineral premix [1]	0.10	0.10	0.08	0.08	0.08
Vitaminic premix [1]	0.05	0.05	0.04	0.04	0.04
Choline chloride 60%	0.05	0.05	0.05	0.05	0.05
Nutricional values (calculated)					
ME (Mcal kg^{-1})	2.96	2.96	3.05	3.05	3.17
Crude protein (%)	22.55	22.55	22.01	22.01	19.10
Dry matter (%)	87.56	87.56	87.85	87.85	87.66
Nutricional values (calculated) (%)					
Lys dig.	1.32	1.32	1.22	1.22	1.10
Met dig.	0.65	0.65	0.58	0.58	0.54
Met+Cys dig.	0.95	0.95	0.88	0.88	0.80
P-total	0.71	0.26	0.64	0.2	0.56
P-available	0.47	0.15	0.41	0.15	0.34
Ca	0.92	0.61	0.84	0.56	0.69
Ca:P	1.95	4.06	2.09	3.75	2.02

[1]Composition (content per kg of diet): 150000 mg of Mn, 100000 mg of Zn, 80000 mg of Fe, 15000 mg of Cu, 1200 mg of I, 700 mg of Se, 23200000 UI of A vitamin, 5600000 UI of D vitamin, 52000 mg of K vitamin, 6000 mg of B1vitamin, 18000 mg of B2 vitamin, 9000 mg of B6vitamin, 132000 mg of niacin, 44000 mg of pantothenic acid, 2400 mg of folic, 200000 µg of biotin, 40000 µg of B12 vitamin

Table 2: Description of the four treatments according to the diets provided from 1-28 days

	Phase and diets		
Treatments	Pre-initial (1-7 days)	Initial (7-21 days)	Growth (21-28 days)
Normal-P (NP)	Control	Control	Control
Depletion (DP)	Control	Reduced	Control
Repletion (RP)	Reduced	Control	Control
Reduced level (RL)	Reduced	Reduced	Control

Reduced phosphorus) in a completely randomized design. Up to 7 days, data were means of 10 pens for broiler chickens fed the pre-starter diet with NP and RL treatments. From 8-28 days, data were means of 5 pens for broiler chickens fed Initial and Grower diets with treatments NP, DP, RP and RL. Means were compared using analysis of variance (ANOVA) followed by Tukey test ($p<0.05$). The program used to perform the data analysis was Minitab (v. 18, Minitab Inc., State College, PA).

RESULTS

Performance evaluation: During the first week of life, the treatments did not affect the BW, FI, BWG and FCR ($p>0.05$; Table 3). Broilers fed the NP diet showed the best results for BW, FI and BWG during the experimental periods of 7-14 and 14-21 days, followed by broilers fed DP and RP diet, while broilers fed the RL diet showed the worst results ($p<0.05$). Broilers fed NP and RP diets showed the best results for BW, FI and BWG during the experimental period of 21-28 days and broilers fed the DP and RL diet presented the worst results ($p<0.05$). The FCR did not differ among treatments in any period ($p>0.05$).

Broilers fed NP and RP diets showed the best results for FI, BWG and FCR during the experimental period of 1-21 days ($p<0.05$). During the whole experimental period (1-28 days), broilers fed NP and RP diets showed the best results for BW, FI and BWG and the best FCR was observed in broilers fed RP

Table 3.Performance of broiler chickens receiving different levels of phosphorus in the diet from 1-28 days of age

	Treatments[1]					
Variables	NP	DP	RP	RL	SEM[2]	p-value
1-7 days[3]						
BW0 (g)	45		45		0.124	ns
BW7 (g)	167		159		1.620	ns
FI (g)	144		136		2.620	ns
BWG (g)	119		113		1.650	ns
FCR (g.g)	1.22		1.20		0.020	ns
7-14 days						
BW14	435^{a}	413^{a}	401^{ab}	364^{b}	7.250	*
FI (g)	374^{a}	332^{ab}	326^{ab}	290^{b}	9.210	*
BWG (g)	270^{a}	251^{ab}	243^{b}	203^{c}	6.450	*
FCR (g.g)	1.38	1.32	1.34	1.42	0.023	ns
14-21 days						
BW21	898^{a}	758^{bc}	810^{ab}	673^{c}	22.800	*
FI (g)	635^{a}	507^{b}	541^{ab}	439^{b}	21.300	*
BWG (g)	463^{a}	345^{c}	409^{b}	309^{c}	16.700	*
FCR (g.g)	1.37	1.46	1.32	1.42	0.014	ns
21-28 days						
BW28 (g)	1569^{a}	1283^{b}	1551^{a}	1209^{b}	37.600	*
FI (g)	956^{a}	750^{b}	964^{a}	720^{b}	24.800	*
BWG (g)	671^{a}	525^{b}	741^{a}	536^{b}	19.500	*
FCR (g.g)	1.42	1.42	1.30	1.34	0.039	ns
1-21 days						
FI (g)	1153^{a}	984^{b}	1003^{a}	864^{b}	30.500	*
BWG (g)	853^{a}	713^{ab}	764^{a}	348^{c}	22.800	*
FCR (g.g)	1.35^{a}	1.38^{a}	1.31^{a}	2.48^{b}	0.011	*
7-21 days						
FI (g)	1009^{a}	839^{b}	867^{b}	729^{c}	30.000	*
BWG (g)	733^{a}	596^{b}	652^{a}	233^{c}	22.200	*
FCR (g.g)	1.38^{a}	1.41^{a}	1.33^{a}	3.13^{b}	0.013	*
1-28 days						
FI (g)	2109^{a}	1734^{b}	1967^{a}	1584^{b}	31.200	*
BWG (g)	1524^{a}	1238^{ab}	1505^{a}	884^{b}	20.100	*
FCR (g.g)	1.38^{b}	1.40^{b}	1.31^{a}	1.79^{c}	0.012	*

Means followed by different letters differ by Tukey's test at 5% probability. *Significant at 5% and ns: non significant. [1]NP: Normal phosphorus, DP: Depletion, RP: Repletion, RL: Reduced level. BW: Body weight, FI: Feed intake, BWG: Body weight gain, FCR: Feed conversion rate, [2]SEM: Standard error of the mean, [3]Up to 7 days, data are means of 10 pens for broiler chickens fed the pre-starter diet with NP and RL treatments. From 8-28 days, data are means of 5 pens for broiler chickens fed Initial and Growth diets with treatments NP, DP, RP and RL

Table 4: Mineral contents of tibia and carcass yield of broilers receiving different levels of phosphorus in the diet from 1-28 days of age

	Treatments					
Variables	NP[1]	DP[2]	RP[3]	RL[4]	SEM[5]	p-value
21 days (%)						
Tibia	40.29^{a}	30.38^{b}	38.82^{a}	31.00^{b}	1.20	*
Carcass yield	72.36	70.02.	72.11	68.74	1.67	ns
28 days (%)						
Tibia	42.27^{a}	33.53^{b}	40,95^{a}	32.47^{b}	1.12	*
Carcass yield	73.48	70.44	74.42	69.65	1.33	ns

Means followed by different letters differ by Tukey's test at 5% probability. *Significant at 5% and ns: Non significant. [1] NP: Normal phosphorus,[2] DP: Depletion,[3] RP:Repletion,[4] RL: Reduced level, [5]Standard error of the mean

diet. Broilers fed DP diet showed the moderate performance and RL showed the worst results for BW, FI, BWG and FCR ($p<0.05$).

Carcass yield, tibia analysis and leg deformities: The carcass yield was not influenced by the treatments in both evaluation ($p>0.05$).The mineral content of tibia was higher in the bones of broilers fed NP and RP diets at 21 and 28 days of life ($p<0.05$, Table 4), while broilers fed DP and RL diets showed low levels of mineral content.

As a complementary result, leg deformities were observed at 28 days when 72 and 48% of the broilers, fed RL and DP

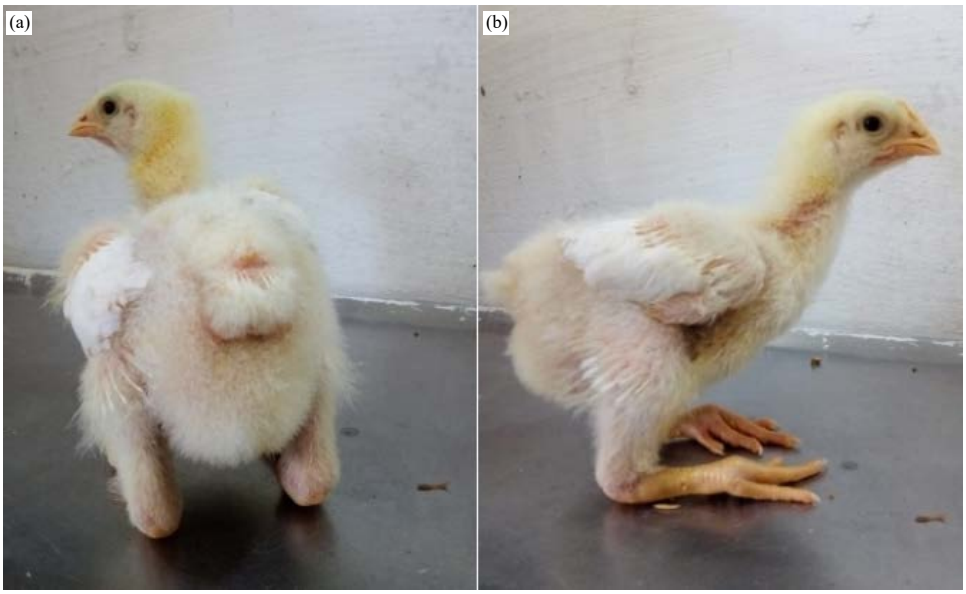

Fig. 1(a-b): Leg disorder observed at 28 days in broilers fed the reduced level diet from 1-21 days of age

diet respectively, showed leg deformities (Fig. 1) and no deformities were observed in boilers fed NP and RP diets.

DISCUSSION

Results demonstrated that the 15% phosphorus reduction upto 21 days of age reduced the performance of broilers. The performance reduction cannot be replenished even when the phosphorus was fed upto the required level after 21 days. On the other hand, broilers fed with 15% phosphorus restricted diet during first week and adequate level in the subsequent period reached to maximum performance.

The normal and reduced level of phosphorus had no effect on performance at 7 days of age. Broilers fed the RL diet showed the worst results for FI and BWG from 8-28 days and the reduced BWG and FI from 14-21 days of age was observed in broilers fed DP diet. These results were reflected in the mineral contents contained in the tibia, which was lower than that of the broilers fed the NP diet at 21 and 28 days. Leg disorders could be observed at 28 days when 72 and 48% of the broilers fed RL and DP diet respectively, presented leg deformities. Studies reported that, P deficient diet can result in the leg disorder like valgus-varus deformity, hypophosphatemic rickets and tibia dyschondroplasia[13,14]. Approximately 80% of the P of the animal organism is constituent of the bones, this mineral is indispensable for the activities of osteoblast and osteocytes in the process of matrix mineralization[15] and the 20% of P that are not in the bones are part of nucleic acids and phospholipids and it is also a co-factor of many enzyme activities[16].

Angel *et al.*[17] observed that nonphytate P (nPP) has a quadratic effect on BWG and the inflection point is between 0.28 and 0.32% of nPP, when broilers from 18-32 days of age fed a diet meeting NRC[18] requirements. Yan *et al.*[7] evaluated the ability of broilers to adapt the moderate reduction of P (0.30%) and found that at 23 days of age, this reduction had no effect on BWG when compared to broilers fed the control diet, results were related to a higher ability to absorb P of broilers fed diets with reduced P. In a meta-analysis, Létourneau-Montminy *et al.*[19] also showed that feeding broilers (21 days) with 3.0 g of nPP/kg of BW did not affect growth performance. However, this supply level can reduce bone mineralization and impair animal welfare[20]. With these results, it is possible to affirm that the reduction of P made in this study (0.15%) is very drastic and the animals can not adapt to this extreme nutritional condition.

Broilers fed RP diet showed 10% reduction in BWG compared to those fed NP diet at 14 days and this lower result was maintained until 21 days. However, from 21-28 days of age, a compensatory growth was observed and similar results of BW, BWG and FI were seen in the end of trial, compared to NP broilers. In addition, broilers fed RP diet showed the best FCR in the whole experimental period (1-28 days). The utilization of metabolizable energy intake for maintenance

(MEm) and productive process in young broilers represents 52-64% of intake[21]. The MEm of broilers is reported as a function of BW raised to the 0.75 power[22,23] and taking into account that broilers fed RP diet were smaller, they needed less energy for maintenance and more energy for weight gain as compared to broilers fed NP diet.

Treatments had negative effect on tibia ash at 21 and 28 days and the broilers fed DP and RL diet showed the worst results of tibia ash. However, broilers fed RP diets showed best results and broilers fed NP diet showed similar results, demonstrating the ability to adapt the severe reduction in the first week of life if fed NP diet during the remaining weeks. It can be associated to the residual yolk sac that provides nutrients, such as phospholipids, to the newly hatched avian during the first few days of life[24]. Bar *et al.*[25] also concluded that broilers exhibit higher capacity of adaptation to P deficient diet in early life and this capacity remains for the whole growth period. Ashwell and Angel[26] reported that broilers fed a moderately deficient P diet in first 90 h were better able to handle a deficiency in the grower phase compared to those fed a control diet in the first 90 h. It indicates that post hatch period changes the broiler's response to diet due to activity of alkaline phosphatase in the tibia and plasma that is increased after hatching and reaches to its peak at 7 days of age[2]. Broilers may respond to a decrease in dietary phosphorus by increasing mRNA expression that encodes phosphorus transporters[26,27] in the small intestine. Several possible mechanisms might be involved in an animal's adaptation to P deficiency and the application of the adaptation principle in poultry must be better studied to further fine tune the degree and period of the restriction with the aim to achieve high productive rates without compromising bone mineralization.

CONCLUSION

It was concluded that reduced indexes of performance and ash content in tibia indicate that broilers have not the ability to adapt the extreme nutritional condition of 0.15% reduction in dietary P, during the initial phase. Broilers may have the ability to adapt the severe reduction of P in the first week of life if fed a diet that meet the nutritional requirements during the remaining weeks.

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