Short communication

Effect of combination of high-dose phytase, citric acid and carbohydrases on performance of broiler chickens fed wheat-canola meal-based diets with very low content of non-phytate phosphorus

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Abstract An experiment was conducted, from 22 to 42-d-post-hatch, to evaluate the effect of different combinations of carbohydrases (CHS), citric acid (CA) and high-dose phytase in wheat-canola meal-based diets with very low content of non-phytate phosphorus (NPP) on growth performance, plasma calcium (Ca) and P and tibia ash (TA) content of broiler chickens. One hundred and ninety two 21-d-old male chicks were allocated into 24 pens and fed one of six dietary treatments. The dietary treatments were T1) a negative control (NC, 1.68 g/kg NPP), T2) NC + CHS (500 mg/kg) + 2,000 phytase FTU/kg, T3) NC + CHS + CA (20 g/kg) + 2,000 phytase FTU/kg, T4) NC + CHS + 4,000 phytase FTU/kg, T5) NC + CHS + CA + 4,000 phytase FTU/kg or T6) a positive control (PC, 4.2 g/kg NPP). The birds fed on PC had higher average daily gain (ADG, $P < 0.01$), average daily feed intake (ADFI, $P = 0.05$), plasma P ($P < 0.001$) and TA content ($P < 0.001$), and lower feed conversion ratio (FCR, $P < 0.05$) and plasma Ca ($P < 0.001$) than those fed on NC. Although the dietary inclusion of additives in NC + CHS + 2000, NC + CHS + CA + 2000, NC + CHS + 4000 and NC + CHS + CA + 4000 significantly improved the growth performance, plasma P and TA content of chicks compared with that of the NC group, all of them could not give rise the results statistically to the similar amount to those of the PC group. NC + CHS + CA + 2000, NC + CHS + 4000 and NC + CHS + CA + 4000 showed similar effects on ADG and plasma P compared to those of the PC group. The data of FCR revealed that NC + CHS + CA + 2000 and NC + CHS + CA + 4000 had a comparable result compared to that of the PC group. NC + CHS + 2000, NC + CHS + CA + 2000, NC + CHS + 4000, NC + CHS + CA + 4000 and PC groups revealed similar effects on ADFI and plasma Ca. The data of TA content showed that NC + CHS + 4000 and NC + CHS + CA + 4000 had similar result compared to that of the PC group. The results showed when diets containing very low content of NPP are supplemented with CA, a combination of phytase (2,000 FTU/kg) and CHS could result in growth comparable to the diet containing adequate NPP.

Keywords: broiler performance, carbohydrases, citric acid, high-dose phytase

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Introduction

Many studies have shown that the efficiency of microbial phytase was improved through using citric acid (CA) and non-starch polysaccharide (NSP)-degrading enzymes (Boling et al., 2000; Snow et al., 2004; Woyengo et al., 2008, 2010) in broiler chicks. It has been reported that CA intensified phytate dephosphorylation under *in vitro* condition (Zyla et al., 1995) and improved the efficiency of phytase as a result of chelating the cations which formed insoluble complexes with phytate, thereby increasing phytate solubility (Maenz et al., 1999). In addition, CA enhanced the dissociation between phytate and minerals because of reduction of the digesta pH (Maenz et al., 1999). The NSP-degrading enzymes (carbohydrases, CHS) increased nutrient utilization in poultry as a result of eliminating the nutrient-encapsulating effect of cell walls and reduction of digesta viscosity (Kim et al., 2005). The CHS might increase the efficacy of phytase because of elimination of phytate-chelating effects of NSP (Kim et al., 2005). In previous studies, some additive responses have been reported with the combination of phytase, CHS and CA (Woyengo et al., 2010) or the combination of phytase and CA (Boling et al., 2000; Snow et al., 2004) in broiler nutrition.

There are three main reasons that justified the present study; a combination of CHS and phytase or a combination of CHS, CA and phytase has not been evaluated in 1) severely limited NPP diets, 2) diets supplemented with high-dose of phytase or 3) wheat-based diets. The high-dose of phytase has been supplemented to the NC diets without any combination with CA or CHS (Shirley and Edwards, 2003; Taheri et al., 2015). Taheri et al (2015) showed that the supplementation of phytase at 1,000 or 2,000 FTU /kg of a corn-soybean meal-based NC diets did not increase the growth performance of the broiler chickens to the similar amount to the positive control. Therefore, in the present study, the effect of supplementing a diet, which was severely limited in NPP (i.e. without any inorganic P supplementation in diet) and based on wheat-canola meal, with CHS + phytase or with CHS + CA + phytase were investigated on growth performance of broiler chickens. Higher feed intake and P excretion from 22 to 42 d of age was the main reason for conducting the experiment in this period.

Materials and methods

This study used 21-d-old Ross 308 broiler chicks and was conducted from 22 to 42 d of age. All birds received a similar diet containing 5.0 and 4.5 g/kg non-phytate P (NPP), respectively, from 1 to 10 and 11 to 21 d of age. Mean body weight of the chicks was similar (700±15 g) in all pens at 22 d of age. The chicks were reared in floor pens under environmentally controlled condition. They were exposed to a 23:1 light:dark cycle and had *ad libitum* access to water and mash diets.

All diets were formulated to meet Ross 308 broiler nutrient requirements of the finisher phase (Ross, 2009) with the exception of NPP in the NC diets (Table 1). One hundred and ninety two 21-d-old male chicks were allocated to one of six wheat-canola meal based dietary treatments in 24 pens. The dietary treatments were T1) a negative control [NC, 1.68 g/kg NPP], T2) NC + CHS + 2,000 phytase FTU/kg, T3) NC + CHS + CA + 2,000 phytase FTU/kg, T4) NC + CHS + 4,000 phytase FTU/kg, T5) NC + CHS + CA + 4,000 phytase FTU/kg or T6) a positive control (PC, 4.2 g/kg NPP). The CHS were the NSP-hydrolysing enzyme multicomplex (Rovabio Excel 10%, Adisseo, France). It was a commercial supplement of *Penicillium funiculosum* products with 2,200 units visco (equivalent to 1400 units AXC) of endo-1,4 beta-xylanase and 200 ACL units of endo-1,4 beta-glucanase per g of the supplement. This supplement was administered at 500 mg/kg of the diet (according to the company's recommendation) in the corresponding treatments (i.e. T2, T3, T4 and T5). The CA was added at a concentration 20 g/kg of the diet in the corresponding treatments (i.e. T3 and T5). Supplemental phytase was a commercially available Natuphos phytase (BASF, Mt. Olive, NJ); prepared from *Aspergillus niger*. The Natuphos phytase had an enzyme activity of 1,000 phytase FTU/g. It was administered at the concentrations 2 and 4 g/kg of the diet, respectively, in treatments T3-T4 (2,000 phytase FTU/kg) and T5-T6 (4,000 FTU/kg).

Birds were weighed on d 21 and 42 to calculate average daily gain (ADG). Feed intake was also measured

Table 1. Ingredients and chemical composition (g/kg) of positive and negative control diets (as fed-basis) from 22 to 42 d of age

Ingredients	PC[a]	NC[a]
Corn (85 g/kg CP)	155	155
Soybean meal (415 g/kg CP)	111.1	111.1
Canola meal (315 g/kg CP)	300	300
Wheat (115 g/kg CP)	300	300
Soybean oil	100	100
Dicalcium phosphate	14	-
Calcium carbonate	7.0	15.3
Sand	-	5.7
Common salt	2	2
Sodium bicarbonate	2.2	2.2
L-Threonine	0.2	0.2
DL-Methionine	1.4	1.4
L-Lysine HCl	2.1	2.1
Vitamin premix[b]	2.5	2.5
Mineral premix[c]	2.5	2.5
Total	1000	1000
Calculated composition[d]		
ME (MJ/kg)	13.45	13.45
Crude protein	192	192
Lysine	10.9	10.9
Methionine+Cystine	8.6	8.6
Threonine	7.5	7.5
Calcium	8.6	8.6
Nonphytate phosphorus	4.2	1.68
Total phosphorus	8.06	5.54
Sodium	1.7	1.7
DCAD[e] (mEq/kg)	217	217

[a]PC = positive control; NC = negative control.

[b]The vitamin premix supplied the following per kg of complete feed: vitamin A, 9,000 IU (retinyl acetate); cholecalciferol, 2,000 IU; vitamin E, 40 IU (dl-α-tocopheryl acetate); vitamin B_{12}, 0.015 mg; menadione, 2 mg; riboflavin, 6.6 mg; thiamine, 1.8 mg; pantothenic acid, 30 mg; niacin, 10 mg; choline, 500 mg; folic acid, 1 mg; biotin, 0.1 mg; pyridoxine, 3 mg.

[c]The mineral premix supplied the following per kilogram of complete feed: manganese ($MnSO_4 \cdot H_2O$), 80 mg; zinc (ZnO), 80 mg; iron ($FeSO_4 \cdot 7H_2O$), 80 mg; copper ($CuSO_4 \cdot 5H_2O$), 10 mg; selenium (Na_2SeO_3), 0.3 mg; iodine (Iodized NaCl), 0.8 mg; cobalt ($CoCl_2$), 0.25 mg.

[d]The ingredients were analyzed for CP, total P and Ca.

[e]DCAD = Dietary cathion-anion difference.

from d 22 to 42 of age and used to calculate average daily feed intake (ADFI) and feed conversion ratio (FCR). On d 42, four birds per replicate (16 birds per treatment) were randomly selected and blood samples were obtained via the wing vein. Plasma was removed and stored at -20°C for further analysis. Concentrations of inorganic P and Ca in plasma samples were measured in duplicate using a spectrophotometer following the instructions of the commercial kits (Pars Azmoon, Iran). On d 42, one bird per replicate (four birds per treatment) was randomly slaughtered to obtain the tibia samples. This tissue was stripped off the bone, and the tibia dried overnight at 100°C, extracted in ether for 6 h, and ashed in a muffle furnace for 15 h at 540°C. Tibial ash (TA) percent was calculated as: [tibia ash weight/dry defatted tibia weight] × 100.

Data were analyzed using the GLM procedure of SAS (SAS, 2003). Pen was the experimental unit, except for tibia ash in which the individual bird was the experimental unit. All statements of significance were based on $P \leq 0.05$. All of the means were compared using least significant difference at P = 0.05.

Results

The results showing the effect of dietary treatments on growth performance, plasma P, plasma Ca and TA content are presented in tables 2 and 3. Overall (d 22 to 42) mortality was approximately less than 3%, and not related to dietary treatments ($P > 0.05$; data not shown). The birds fed on PC had higher ADG ($P < 0.01$), ADFI ($P = 0.05$), plasma P ($P < 0.001$) and TA content ($P < 0.001$), lower FCR ($P < 0.05$) and plasma Ca ($P < 0.001$) than those fed on NC. Although the dietary inclusion of additives in NC + CHS + 2000, NC + CHS + CA + 2000, NC + CHS + 4000 and NC + CHS + CA + 4000 significantly improved the growth performance, plasma P and TA content of chicks compared with that of the NC group, all of them could not give rise the results statistically to the similar amount to those of the PC group. NC + CHS + CA + 2000, NC + CHS + 4000 and NC + CHS + CA + 4000 showed similar effects on ADG and plasma P compared to those of the PC group. The data of FCR revealed that NC + CHS + CA + 2000 and NC + CHS + CA + 4000 had a comparable result compared to that of the PC group. NC + CHS + 2000, NC + CHS + CA + 2000, NC + CHS + 4000, NC + CHS + CA + 4000 and PC groups revealed similar effects on ADFI and plasma Ca. The data of TA content showed that NC + CHS + 4000 and NC + CHS + CA + 4000 had similar result compared to that of the PC group.

Discussion

The results showed when diets containing very low content of NPP are supplemented with CA, a combination of phytase (2,000 FTU/kg) and CHS could result in growth comparable to the diet containing adequate NPP. In agreement with the findings of current study, Snow et al. (2004) reported the weight gain of broilers was improved as a result of addition of CA to a phytase (300 U/kg)-supplemented low-NPP diet. In the present study, it had been assumed that high phytate content of canola meal might increase diets' potential responding more to lower doses of phytase supplementation, but the results of ADG and FCR in wheat-canola meal-based dietary treatments of the current experiment were similar to those obtained from soybean meal-based diets (Taheri et al., 2015) which contained lower level of phytate. This might be related to this fact that phytase more readily hydrolyses phytate in soybean meal (72.4%) than canola meal (55.8%), although the one latter contains higher levels of phytate (Leske and Coon, 1999). On the other hand, Liu et al. (2014) declared that phytase impr-

Table 2. Effect of combination of high-dose phytase, citric acid and carbohydrases on growth performance[1] (from 22 to 42 d of age) of male broilers fed wheat-canola meal-based diets

Treatments	Basal diets[2]	Carbohydrases	Citric acid	Phytase (FTU /kg)	ADG (g)	ADFI (g)	FCR
T1	NC	-	-	-	73.4^{c}	121.3^{b}	1.66^{c}
T2	NC	+	-	2000	85.1^{b}	135.2^{a}	1.59^{b}
T3	NC	+	+	2000	89.7^{ab}	137.0^{a}	1.54^{ab}
T4	NC	+	-	4000	88.7^{ab}	140.3^{a}	1.58^{b}
T5	NC	+	+	4000	91.4^{a}	142.3^{a}	1.55^{ab}
T6	PC	-	-	-	90.1^{a}	135.3^{a}	1.51^{a}
SEM					1.73	2.91	0.023
P value					0.004	0.05	0.02

$^{a-c}$In each column, means with no common letter are significantly different (LSD, $P \leq 0.05$).

[1]Broiler chicks were on trial from 22 to 42 d of age. ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

[2]PC = positive control (4.2 g/kg nonphytate phosphorus); NC = negative control (1.68 g/kg nonphytate phosphorus).

Table 3. Effect of combination of high-dose phytase, citric acid and carbohydrases on calcium (Ca) and phosphorus (P) of plasma and tibia ash (at d 42) in male broilers[1] fed wheat-canola meal-based diets

Treatments	Basal diets[2]	Carbohydrases	Citric acid	Phytase (FTU /kg)	Ca (mg/dL)	P (mg/dL)	Tibia ash (%)
T1	NC	-	-	-	12.5[a]	3.1[e]	42.1[d]
T2	NC	+	-	2000	9.7[b]	5.2[d]	43.9[c]
T3	NC	+	+	2000	10.5[b]	6.50[c]	44.8[bc]
T4	NC	+	-	4000	9.7[b]	7.2[a]	45.9[ab]
T5	NC	+	+	4000	9.5[b]	7.0[ab]	46.6[a]
T6	PC	-	-	-	9.5[b]	6.8[abc]	46.7[a]
SEM					0.35	0.14	0.55
P value					0.0001	0.0001	0.0005

[a-e] In each column, means with no common letter are significantly different (LSD, $P \leq 0.05$).

[1]Broiler chicks were on trial from 22 to 42 d of age.

[2]PC = positive control (4.2 g/kg nonphytate phosphorus); NC = negative control (1.68 g/kg nonphytate phosphorus).

oved weight gain and FCR in corn-, sorghum- and wheat-based diets with more pronounced response in corn-based diets. In addition, phytase also significantly enhanced nutrient utilization in corn-based diets, but not in sorghum- or wheat-based diets.

A deficiency of NPP in chickens was characterized by a reduction in feed intake and reduced circulating levels of growth hormone (Parmer et al., 1987). Although there were some studies that showed no significant difference in feed intake when phytase was supplemented to NPP deficient diets (Pirgozliev et al., 2010; Shaw et al., 2011; Chung et al., 2013; Walk et al., 2013), other researchers found that feed intake was influenced when phytase was supplemented to the negative control (Shirley and Edwards 2003; Han et al., 2009; Liu et al., 2010; Rutherfurd et al., 2012; Pirgozliev and Bedford, 2013; Walk et al., 2012, 2014; Zyla et al., 2013). Also, the beneficial effect of high-dose phytase on feed efficiency or weight gain was observed when supplemented to soybean meal-based diets limited in NPP (Liu et al., 2010; Pirgozliev and Bedford, 2013; Shaw et al., 2011). However, several studies showed no significant effect on feed efficiency when phytase was supplemented to a negative control (Pirgozliev et al., 2007; Rutherfurd et al., 2012; Gehring et al., 2013). Although the beneficial effect of CA on weight gain or feed intake has been also observed in the literature (Boling et al., 2000; Snow et al., 2004), its combination with phytase did not increase ADFI in the present study. The reason of this discrepancy is not clear.

Similar to ADG, ADFI, and FCR, principal improvements in plasma Ca and plasma P data were indicative of better phytate utilization. The results showed when diets containing very low content of NPP are supplemented with CA, a combination of phytase (2,000 FTU/kg) and CHS could result in growth comparable to the diet containing adequate NPP. The effect of phytase supplementation on plasma P has been reported in the previous researches (Shirley and Edwards, 2003; Han et al., 2009; Liu et al., 2010). Shirley and Edwards (2003) showed that phytase supplementation only at 12,000 FTU/kg in NPP deficient corn-soybean meal diets enhanced plasma P comparable to the PC. Although the mechanisms including the reduction of anti-nutritional effects of phytate and inositol effects of phytase superdoses might be involved in improvement of broiler's performance (Li et al., 2000; Cowieson et al., 2006a,b; Cowieson et al., 2011; Walk et al., 2014), the improved weight gain and feed efficiency were interpreted as a phytase induced release of phytate-bound P and increasing the plasma P. There was a relationship between plasma P and weight gain in the literature (Shirley and Edwards, 2003). The ability of phytase to improve P availability by hydrolyzing phytate-bound P in poultry diets was well documented (Kornegay et al., 1996a,b; Qian et al., 1997). In addition, high levels of dietary inclusion of phytase have shown phytate disappearance from the digesta of broiler chicks with each additional dose of phytase (Shirley and Edwards, 2003; Han et al., 2009). Additionally, it has been shown that the supplementation of CA in broiler chicken diets enhanced the efficiency of phytase in hydrolyzing phytate (Woyengo et al., 2010). As it was explained before, CA chelated cations that formed insoluble complexes with phytate, thereby increasing phytate solubility (Maenz et al., 1999). In addition, it has been shown that CA reduced the pH of the digesta (Radcliffe et al., 1998), increased the dissociation between phytate and minerals (Maenz et al., 1999) and enhanced the activity of phytase, which expressed its optimal activity at low pH (Simon and Igbasan, 2002). These mechanisms could be the explanation for the obtained results by T3 than T5 in the present study.

For Ca, similar results have been found by Viveros

et al. (2002) and Shirley and Edwards (2003). A low-P plasma concentration led to the activation of vitamin D in the kidney that, in turn, led to increased P absorption and reabsorption in the small intestine and kidney, respectively (Proszkowiec-Weglarz and Angel, 2013). At the same time, bone resorption was induced to maintain a normal plasma P concentration (Proszkowiec-Weglarz and Angel, 2013), thereby increasing plasma P and Ca. It seems the amount of released P from bones could not compensate P deficiency and increase plasma P; however, the released Ca from bone resorption increased plasma Ca, because there was no Ca deficiency in the diet or plasma at the same time. A high plasma Ca was not related to the increased digestibility of diet Ca, because low NPP diets even have decreased Ca retention in the gut (Sebastian et al., 1996; Viveros et al., 2002; Shirley and Edwards, 2003). In agreement with the results of present study, Viveros et al. (2002) and Shirley and Edwards (2003) found the decrease in plasma Ca concentrations with phytase addition. Shirley and Edwards (2003) found that graded levels of phytase had large effects on phytate disappearance, total P retention and plasma P values, but showed little effect on plasma Ca or the total Ca retention values. In the study of Shirley and Edwards (2003), higher levels of phytase supplementation even tended to slightly increase the retention of dietary Ca as the concentration of plasma Ca decreased. Nevertheless, there were other studies that did not show any significant effect of phytase on plasma Ca concentration (Roberson and Edwards, 1994; Rama-Rao et al., 1999).

The reduced TA in the NC diet might be related to the lower plasma P and higher bone resorption than those of the PC (Taylor and Dacke, 1984). The increased plasma P might be the cause of the improved TA in the NC diets supplemented with phytase at 4,000 FTU/kg (with or without CA) or 2,000 U/kg plus CA. Other studies also have shown that bone ash content was increased by graded levels of phytase supplementation (Dilger et al., 2004; Jendza et al., 2006; Han et al., 2009; Shaw et al., 2011; Rutherfurd et al., 2012; Walk et al., 2012, 2013, 2014; Zhang et al., 2000). However, the present study showed that the supplementation of CA reduced the need of phytase in order to increase TA content of the NC diets.

Conclusion

The results showed when diets containing very low content of NPP are supplemented with CA, a combination of phytase (2,000 FTU/kg) and CHS could result in growth comparable to the diet containing adequate NPP.

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اثر تلفیق سطوح بالای فیتاز، اسید سیتریک و کربوهیدراز روی عملکرد جوجه‌های گوشتی تغذیه شده با جیره‌های بر پایه گندم–کنجاله کانولای حاوی فسفر غیرفیتاته بسیار پایین

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چکیده آزمایشی از ۲۲ تا ۴۲ روزگی به منظور بررسی اثر تلفیق‌های مختلف کربوهیدراز، اسید سیتریک و سطوح بالای فیتاز در جیره‌های بر پایه گندم- کنجاله کانولای حاوی فسفر غیرفیتاته بسیار پایین روی عملکرد، کلسیم و فسفر پلاسما و خاکستر در شت‌نی جوجه‌های گوشتی طرح‌ریزی شد. ۱۹۲ جوجه نر ۲۱ روزه به ۲۴ پن و یکی از ۶ جیره آزمایشی اختصاص داده شد. جیره‌های آزمایشی عبارت بودند از جیره ۱) کنترل منفی (با ۱/۶۸ گرم فسفر غیرفیتاته در کیلوگرم)، جیره ۲) کنترل منفی + کربوهیدراز (۵۰۰ میلی‌گرم در کیلوگرم) + ۲۰۰۰ واحد فیتاز در کیلوگرم، جیره ۳) کنترل منفی + کربوهیدراز + اسید سیتریک (۲۰ گرم در کیلوگرم) + ۲۰۰۰ واحد فیتاز در کیلوگرم، جیره ۴) کنترل منفی + کربوهیدراز + ۴۰۰۰ واحد فیتاز در کیلوگرم، جیره ۵) کنترل منفی + کربوهیدراز + اسید سیتریک + ۴۰۰۰ واحد فیتاز در کیلوگرم، و جیره ۶) کنترل مثبت (با ۴/۲ گرم فسفر غیرفیتاته در کیلوگرم). پرندگان تغذیه شده با جیره کنترل مثبت دارای میانگین افزایش وزن روزانه ($P < 0.01$)، میانگین مصرف خوراک روزانه ($P = 0.05$)، فسفر پلاسما ($P < 0.001$) و محتوای خاکستر درشت‌نی ($P < 0.001$) بالاتر، و ضریب تبدیل خوراک ($P < 0.05$) و کلسیم پلاسمای ($P < 0.001$) پایین‌تری نسبت به پرندگان تغذیه شده با جیره کنترل منفی بودند. اگرچه استفاده از افزودنی‌ها در کنترل منفی + کربوهیدراز + ۲۰۰۰، کنترل منفی + کربوهیدراز + اسید سیتریک + ۲۰۰۰، کنترل منفی + کربوهیدراز + ۴۰۰۰، و کنترل منفی + کربوهیدراز + اسید سیتریک + ۴۰۰۰ به طور معنی‌داری عملکرد رشد، فسفر پلاسما و محتوای خاکستر درشت‌نی را در مقایسه با گروه کنترل منفی بهبود دادند، اما همه آنها نتوانستند نتایج را از لحاظ آماری به سطح مقادیر مشابه با گروه کنترل مثبت برسانند. کنترل منفی + کربوهیدراز + اسید سیتریک + ۲۰۰۰، کنترل منفی + کربوهیدراز + ۴۰۰۰ ، و کنترل منفی + کربوهیدراز + اسید سیتریک + ۴۰۰۰ در میانگین افزایش وزن روزانه و فسفر پلاسما اثرات مشابهی در مقایسه با گروه کنترل مثبت نشان دادند. نتایج ضریب تبدیل خوراک نشان داد که کنترل منفی + کربوهیدراز + اسید سیتریک + ۲۰۰۰، و کنترل منفی + کربوهیدراز + اسید سیتریک + ۴۰۰۰ دارای اثرات مشابهی با گروه کنترل مثبت هستند. کنترل منفی + کربوهیدراز + ۲۰۰۰، کنترل منفی + کربوهیدراز + اسید سیتریک + ۲۰۰۰، کنترل منفی + کربوهیدراز + ۴۰۰۰، کنترل منفی + کربوهیدراز + اسید سیتریک + ۴۰۰۰ و گروه کنترل مثبت نتایج یکسانی را در میانگین مصرف خوراک روزانه و کلسیم پلاسما نشان دادند. نتایج محتوای خاکستر درشت‌نی نشان داد که کنترل منفی + کربوهیدراز + ۴۰۰۰، و کنترل منفی + کربوهیدراز + اسید سیتریک + ۴۰۰۰ دارای اثرات مشابهی در مقایسه با گروه کنترل مثبت هستند. نتایج نشان داد هنگامی که جیره‌های حاوی سطوح بسیار پایین فسفر غیرفیتاته با اسید سیتریک مکمل شوند تلفیقی از کربوهیدراز و ۲۰۰۰ واحد فیتاز در کیلوگرم توانست عملکرد رشد مشابهی را در مقایسه با جیره حاوی سطوح کافی فسفر غیرفیتاته ایجاد کند.