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Influence of calcium and non-phytate phosphorus deficient diets with phytase on the performance of broilers, serum concentrations of minerals and the activity of alanin transaminase, aspartate transaminase and lactate dehydrogenase

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Abstract: **An experiment was conducted to study the effect of microbial phytase (phyzyme XP5000G) supplementation in broiler chicks' diets on the performance, serum total protein (TP), minerals (Ca, Mg and P) and the serum enzyme activities (AST, ALT, LDH). A reference diet adequate in calcium and non-phytate phosphorus (10.0 gkg^{-1} Ca and 5 gkg^{-1} nPP) and two deficient diets in Ca and nPP (8.5 gkg^{-1}Ca and 3.5 gkg^{-1} nPP, and 7.5 gkg^{-1} Ca and 2.5 gkg^{-1} nPP) with or without phytase (0 and 100$mgkg^{-1}$) were offered to broiler chicks from 1 to 21 days of age. Although the low-nPP diets had no significant effect on body weight gain (BWG) of chicks ($p>0.05$) They increased ($p<0.01$) feed intake (FI) and feed conversion ratio (FCR) when compared to the low-nPP diet supplemented with enzyme. Phytase had a favorable effect, although non-significantly, on BWG of chicks fed very low level of nPP. Enzyme reduced the feed intake ($p<0.05$) and improved the FCR of Ca-nPP deficient chicks ($p<0.01$). The decrease in Ca-nPP content in the diet caused a significant increase in serum concentration of Ca ($p< 0.05$) and decrease in P concentration ($p< 0.05$). Low Ca-nPP diets had no influence on serum Mg concentration. Dietary phytase reduced the Ca level and increased the P level ($p<0.05$) of serum in chicks fed with Ca-nPP deficient diets. The activity of LDH increased ($p<0.01$) in response to low dietary Ca and nPP, deficient but there was no influence on serum ALT and AST activity and TP content ($p>0.05$). Phytase supplementation reduced serum ALT ($p<0.05$) and had no effect on AST ($p>0.05$). Serum LDH activity further increased ($p<0.01$) by phytase supplementation. These results demonstrated that the performance of the chicks received low levels of Ca and nPP with phytase was improved and the serum parameters were comparable to those chicks fed with normal Ca-nPP diet.**

Key words: phytate phosphorus, phyzyme, broiler performance.

Introduction

Most cereal grains and oilseeds contain phytin between 0.7% and 2%, which serves as the storage form of phosphorus, representing 50% to 85% of the total P (Cheryan 1980).

Dietary phytin P is poorly digested by monogastric animals, as seen in the excretion of considerable quantities of P in manure from intensive operations. The poor digestive utilization of phytin-bound P by poultry and its consequences on diet cost,

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environment, and digestibility of minerals and proteins have led to extensive research efforts directed toward understanding the process of phytic acid digestion.

There are several reviews on nutritional (Cheryan, 1980, Ravindran *et al.*, 1995, Selle *et al.*, 2000, Adeola and Sands 2003) and environmental (Jongbloed and Lenis, 1998) consequences of phytin, as well as the application, structure, and kinetic properties of phytase (Dvorakova, 1998; Liu *et al.*, 1998; Keshavarz, 2000; Yu *et al.*, 2004). Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. The enzyme is at low level in the chicken gastro-intestinal tract but is present in some cereals such as wheat and triticale and at high concentration in microbial sources. Supplementation of diets with microbial phytase has proven to be an effective and realistic method for enhancing the digestibility of phytic acid (Nys *et al.*, 1999). Phytase reduces the need for supplemental inorganic P to the diet and hence, the amount of P excreted into the environment (Coelho, 1999, Kies, 1999, Selle *et al.*, 2000, Kies *et al.*, 2001, Adeola and Sands 2003). However, little is known about the effect of diets deficient in Ca and nPP, and with phytase on serum enzyme activity of broiler chicks. Therefore, the objective of this experiment was to evaluate the effect of phytase on body weight gain, feed intake, feed conversion ratio, and serum concentrations of Ca, P, Mg and total proteins as well as the activity of enzymes AST, ALT and LDH, in corn-soy bean meal based diets deficient in Ca and nPP compared to a nutritionally adequate diet for broiler chicks.

Materials and Methods

Six hundred 1-day old Ross male, broiler chicks (43±1.5g BW) were allotted to five treatments in a completely randomized design. Each treatment was replicated four times with thirty chicks in each replicate. The chicks were provided the experimental diets and the experiment lasted for 21 days. The birds were housed in an environmentally controlled room with raised wire floors. They were exposed to continuous light for the first 2 days, then to 23 h light and 1h darkness until 21 days of age. Chicks, feed, and water were checked twice daily, and feed in the mash form and water was provided on an ad libitum basis throughout the experiment.

A corn- soybean meal diet (C-SBM), adequate in all nutrients (except Ca and nPP) for experimental purposes was used. The diet (Table 1) provided $220gkg^{-1}$ crude protein and 2900 kcal $MEkg^{-1}$, and met all other nutrient requirements (NRC, 1994). The dietary treatments were: 1) C-SBM, $10gkg^{-1}$ Ca and $5gkg^{-1}$ nPP (Diet 1); 2) C-SBM, $8.5gkg^{-1}$ Ca and $3.75gkg^{-1}$ nPP (Diet 2); 3) C-SBM, $7.5gkg^{-1}$ Ca and $2.5gkg^{-1}$ nPP (Diet 3); 4) Diet 2 + $100mgkg^{-1}$ phytase (Phyzyme XP5000G, Danisco Animal Nutrition, Marlborough, Wiltshire, SN8 1XN, UK); 5) Diet 3 + $100mgkg^{-1}$ phytase. The amount of enzyme added to the diet was based on the manufacturer's recommendations.

Growth Performance

At the end of experimental period, the birds were weighed and feed consumption was recorded for feed efficiency computation. Feed conversion ratio (FCR) was calculated from the ratio of feed intake (FI) to body weight gain (BWG) for the 21 days of experimental treatment.

Serum Ca, P, Mg, ALT, AST, LDH and TOP

At 21 days of age, two birds were randomly selected from each pen and blood samples (10ml) were obtained by bleeding. Blood tubes were centrifuged at 1200 ×g and supernatants were collected in 1.5 ml Ependorf tubes and deep frozen (-20 °C) until measurement of minerals (Ca, P, Mg), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and total protein (TOP) in serum using commercial diagnostic kits and according to the standard procedures (Eppendorf Blood Analyzer, Model EPOS 5060, Netheler-Hinz GmbH, Hamburg, Germany) (AOAC, 1990).

Statistical Analysis

The experiment had a complete random design (ANOVA) (Minitab 13.2 statistical package, Minitab Inc. State College) with a 2 ×2 factorial arrangement (two diets deficient in Ca and nPP and two levels of enzyme) with a reference diet adequate in Ca and nPP with no enzyme. Fisher's LSD method was used to find confidence intervals for all pair wise differences between means. The general linear model was used to determine the main effects of factors and any possible interaction between factors.

Results

The influence of phytase enzyme on body weight gain, feed intake, feed conversion ratio of the chicks and the concentration of Ca, Mg, P and TP in serum and the activity of enzymes ALT, AST and LDH, is shown in Table 2.

There was no significant difference ($p>0.05$) in BWG of chicks among treatments. However, those chicks offered with Ca-nPP deficient diets had lower BWG values than the Ca-nPP adequate diet. Adding phytase tended to increase the BWG of Ca-nPP deficient groups to the level of Ca-nPP adequate diet.

There was a numeric increase in feed intake of the birds who received the lowest level of Ca-nPP diet compared to Ca-nPP adequate birds (30g), but phytase reduced significantly ($p<0.05$) the amount of feed consumed by those chicks fed the diet with the lowest level of Ca-nPP by almost 100g.

Those birds offered with the lowest level of Ca-nPP diet had the highest FCR and the birds received the same diet with enzyme had the lowest FCR among all treatments ($p<0.01$).

The serum concentration of Ca decreased and concentration of P increased when the level of Ca and P in the experimental diet decreased ($p<0.05$). However, the serum concentrations of Ca and P in birds provided with the same diets containing enzyme, were comparable to Ca-nPP adequate diet.

Different dietary treatments had no effect on serum concentrations of Mg and TOP, and serum activity of AST.

The serum activity of ALT decreased and the activity of LDH increased ($p<0.05$) in response to the diets deficient in Ca-nPP. Phytase had no influence on ALT activity in Ca-nPP deficient birds ($p>0.05$). However the enzyme further increased the activity of LDH in Ca-nPP deficient groups ($p<0.01$).

Discussion

Almost 70-80% of the phosphorus in feedstuffs of plant origin and 61-70% phosphorus found in corn-soybean meal diets is in the form of phytate phosphorus (Kies *et al*., 2001, Naher 2002). The inability of poultry to utilize phytate phosphorus, due to lack of endogenous phytase, results in the addition of inorganic feed phosphates to poultry diets in order to meet the phosphorus requirements of poultry (Maenz and Classen 1998, Yu, *et al*., 2004).

The adverse effects of phytate phosphorus could possibly be overcome by dietary supplementation of exogenous phytases. Addition of exogenous phytases to the diet has been reported to improve growth performance of broilers (Ahmad *et al*., 2000, Naher 2002) and also improve phosphorus digestibility for certain plant based feedstuffs and overall utilization of the broiler diets (Rutherfurd *et al*., 2002). An addition of phytase to diets reduces the excretion of P in manure and helps to correct the possible environmental pollution (Simons *et al*., 1990, Roberson and Edwards 1994, Kornegay and Harper 1997, Coelho 1999, Kies 1999, Selle *et al*., 2000, Kies *et al*., 2001, Adeola and Sands 2003).

As expected, a decrease in dietary calcium and available phosphorus caused a negative effect on overall performance of chicks. These results were in agreement with those reported by others (Qian *et al*., 1996, Punna and Roland 1999, Rama Rao *et al*., 1999, Yu *et al*., 2004). Similarly, the positive effect of phytase on performance of chicks seen in this experiment, has already been reported by many workers (Broz *et al*., 1994, Sebastian *et al*., 1996, Rama Rao *et al*., 1999, Ahmad *et al*., 2000, Yu *et al*., 2004). As shown here and indicated by others, microbial phytase seems to be effective in releasing Ca and P in diets deficient in Ca and P (Qian *et al*., 1996, Gordon and Roland 1998, Cabahug *et al*., 1999, Sohail and Roland, 1999). The beneficial effect of phytase on growth performance has been related to (1) the release of minerals and trace elements from complexes with phytic acid, and increase in digestibility and availability of macro- and micro-elements (Knuckles and Betschsrt 1987, Qian *et al*., 1996, Sebastian *et al*., 1996, Selle *et al*., 2000, Adeola and Sands 2003), (2) the utilization of inositol (Simons *et al*., 1990), (3) increased starch digestibility (Knuckles and Betschart 1987) and (4) increased utilization of protein (Farrell *et al*., 1993).

In the present study, reducing the Ca and nPP content of diet from 10 to 7.5gkg^{-1} and from 5 to 2.5 gkg^{-1} depressed numerically BDG of the birds, and dietary phytase showed a numeric improvement on the BWG. The results fully agreed with the reports indicating that the increase in concentration of phytase in SBM based diets might increase the body weight of chicks (Salobir *et al*., 1998, Rama Rao *et al*., 1999, Ahmad *et al*., 2000, Moshad 2001). However, Mohanna and Nys (1999), Wilson *et al*., (1999) and Pizzolante *et al*., (2002) did not notice a positive influence of phytase on body weight gain of the experimental birds.

An increase in FI of the chicks offered the lowest level of Ca-nPP in their diet might be in order to compensate factors influencing the need for calcium and phosphorus. This result is in contrast with many workers who demonstrated that a reduction in Ca and nPP of diet reduced the feed consumption of the chicks (Sohail and Roland 1999, Boling *et al*., 2000, Johnston and Southern 2000, Watson 2002). Juanpere *et al*., (2004) showed that a reduction in nPP of broiler chick diets decreased daily feed intake and water consumption and a reduction in bird growth.

On the other hand, in the experiment presented here, a decrease in feed intake of the birds offered the Ca-nPP deficient diets with phytase was probably due to the better digestion and utilization of dietary nutrients. However, this result is in contrast with the results of Denbow *et al*., (1995) and Sebastian *et al*., (1996, 1997), Sohail and Roland (1999), Boling *et al*., (2000), Johnston and Southern (2000), Watson (2002), Brenes (2003) and Juanpere *et al*., (2004). The above authors suggested that the increase in BWG of chicks feed with the dietary phytase was due to an increase in FI.

An improvement in FCR of the birds by the addition of phytase, was similar to those reported by Broz *et al*., (1994), Kornegay *et al*., (1996), Gordon and Roland (1998), Rama Rao *et al*., (1999), Waldroup *et al*., (2000) and Yan *et al*., (2001).

An investigation was carried out by Ahmed *et al*., (2004) on the performance of broiler chicks who were fed soybean meal (SBM) based diet incorporating phytase with the levels of 0.0, 0.50, 1.00 and 1.50 gkg^{-1} diet for better utilization of the basal diet. It was noted that the growth rate, feed intake, feed consumption, dressing yield and profitability increased as the level of phytase supplementation increased. It was suggested that 1.50gkg^{-1} phytase may be incorporated in SBM based broiler diets in order to overcome effectively overcome the antinutritive effect of phytate phosphorus on broiler performance.

An increase in serum concentration of Ca and a decrease in concentration of P in response to decreasing Ca and nPP levels of the diet, has also been observed in chickens and turkeys (Fernandes *et al*., 1999, Atia *et al*., 2000, Brenes *et al*., 2003). The increase in serum Ca level of experimental chicks in response to decreasing dietary Ca and nPP level, might be related to an increase in Ca retention, which has been also indicated by others (Taylor and Dacke 1984, Sebastian *et al*., 1996, Fernandes *et al*., 1999). An increase in P retention in broilers and pullets fed low nPP diets was related to a decrease in plasma P, indicating that birds had a greater ability to retain P from diets with lower nPP content (Ravindran *et al*., 2000, Keshavarz 2000).

In this study, phytase supplementation to the low Ca-nPP diets reduced Ca and increased P concentrations in serum. Similar results have also been observed by Sebastian *et al*., (1996), Han *et al*., (1997), Orban *et al*., (1999), Rama Rao *et al*., (1999) and Hassen and Chauhan (2003), Brenes *et al*., (2003) and Juanpere *et al*., (2004). Perney *et al*., (1993) and Ahmad *et al*., (2000) indicated that the decreased P excretion in birds fed low-P diets with phytase, might be due to the increased bioavailability of both P and Ca, because both are part of the same complex and are released by the phytase enzyme at the same time. When birds were fed diets with phytase, the phosphate group of phytate was degraded and absorbed, and then used for growth. The increased bioavailability of dietary P by adding phytase has also

Table 1: Formulation of diets and chemical composition of control and experimental rations

Ingredients (gkg^{-1})	Treatments		
	Diet 1	Diet 2	Diet 3
Corn	533	547	560
Soybean Meal	394	392	390
Vegetable Fatty Acid	30.4	24.6	20.0
Di-Calcium Phosphate	21.4	14.2	7.1
Limestone	10.1	10.5	11.3
DL-Methionine	1.8	1.8	1.8
L-Lysine	0.2	0.2	0.2
Salt	4.0	4.0	4.0
Vitamin + Mineral Premix*	5.0	5.0	5.0
ChemicalAnalysis (calculated)			
Metabolizable Energy ($kcalkg^{-1}$)	2900	2900	2900
Crude Protein	220	220	220
Crude Fiber	41.0	41.1	41.2
Total Fat	49.4	43.1	40.5
Arginine	15.8	15.8	15.8
Lysine	13.0	13.0	13.0
Methionine	5.4	5.4	5.4
Methionine + Cystine	9.2	9.2	9.2
Threonine	9.0	9.0	9.0
Tryptophan	2.7	2.7	2.7
Total Calcium	10	8.5	7.5
Total Phosphorus	8.0	6.75	5.50
Non-Phytate Phosphorus	5.0	3.75	2.5

*, The vitamin and mineral premix supplied per kg of diet; Vit A 9000IU, Vit D3 2000IU, Vit E 18IU, Vit B1 1.8mg, Vit B2 6.6mg, Vit B3 (Calcium Panthotenate) 10mg, Vit B5 (Niacin) 30mg, Vit B6 3mg, Vit B12 0.015mg, Biotin 0.1mg, Folic Acid 1.0mg, Cholin Chloride 500mg, Vit K 2mg, Manganese 99.2mg, Iron 50mg, Zinc 84.7mg, Copper 10mg, Iodine 1mg, Selenium 0.2mg.

been reported by Nys *et al*., (1999) and Leske and Coon (1999).

Dietary phytic acid might increase the excretion of endogenous compounds by broilers. However, the supplementation of phytase seems to reduce the excretion of compounds with endogenous origin (Cowieson *et al*., 2004).

In the present study, the concentration of total protein and magnesium were not affected either by the dietary concentration of Ca and nPP or by the presence of phytase in the diet. However, it has been documented that the serum total protein and magnesium levels significantly increased when the diet of chicks was supplemented with dietary phytase (Hassen and Chauhan 2003, Brenes *et al*., 2003).

In an experiment by Brenes *et al*., (2003), it was demonstrated that by decreasing the nPP levels in the diet, plasma Ca concentration as well as AST, ALP, and LDH activities were increased and plasma P and TOP content were reduced. Phytase supplementation increased linearly plasma Ca, P, Mg, and TOP content, and serum AST, ALT and LDH activities, but reduced linearly serum ALP activity.

A difference in an activity of serum ALT in response to Ca-nPP deficient diets demonstrated in here and reported by Brenes *et al*., (2003), could be due to the higher activity of enzyme ALT reported here. Adding phytase to the diet could not elevate the activity of the enzyme in this experiment, which is in contrast with their report.

The activity of enzyme AST was not affected either by changes in the dietary concentration of Ca

Table 2: The influence of Phytase on growth performance, serum protein (TOP), minerals (Ca, P, Mg) and enzyme activity (ALT, AST, LDH) of chicks fed Ca-P deficient diets up to 21 days of age.

	Diet 1	Diet 2	Diet 3	Diet 2+ Enz	Diet 3+ Enz	Pooled SEM	Diet	Enzyme	Diet ×Enz
BWG[1] (g)	725	720	713	715	724	18.2	NS	NS	NS
FI[2] (g)	1033^{ab+}	1021^{ab}	1063^{b}	997^{a}	994^{a}	24.2	NS	*	NS
FCR[3]	1.346^{a}	1.340^{a}	1.407^{b}	1.318^{a}	1.300^{a}	0.0179	NS	**	NS
Ca (mg/dl)	7.114^{a}	7.617^{a}	8.729^{b}	7.840^{a}	7.486^{a}	0.2569	*	NS	NS
P (mg/dl)	6.971^{a}	6.217^{ab}	5.350^{b}	6.660^{a}	6.367^{ab}	0.3118	*	NS	NS
Mg (mg/dl)	2.483	2.366	2.554	2.454	2.596	0.1204	NS	NS	NS
TOP[4] (g/100ml)	3.029	2.801	2.823	2.798	2.977	0.0993	NS	NS	NS
ALT[5] (U/L)	13.75^{a}	13.71^{a}	12.63^{ab}	13.43^{a}	11.5^{b}	0.53	*	NS	NS
AST[6] (U/L)	284	243	252	254	275	7.4	NS	NS	NS
LDH[7] (U/L)	2535^{a}	2831^{ab}	3170^{b}	3773^{c}	3843^{c}	181.8	*	**	NS

+, Values within rows with different superscripts are significantly different ($p<0.05$)

NS, Not Significant; *, $p<0.05$; **, $p<0.01$

1, Body Weight Gain; 2, Feed Intake; 3, Feed Conversion Ratio; 4, Total Protein; 5- Alanine Aminotransferase; 6, Aspartate Aminoteransferase; 7, Lactate Dehydrogenase

and nPP or by supplementation of dietary phytase, which is in agreement with the results of Kundu *et al.*, (2004).

Viveros *et al.*, (2002) informed that by decreasing nPP levels of the diet, plasma Ca and Mg concentrations increased, and plasma P and TOP content as well as AST activity were reduced. Phytase supplementation increased plasma P level and serum AST activity, reduced plasma Ca and Mg contents and reduced serum ALT and LDH activities.

An elevation in activity of enzyme LDH in response to the low levels of Ca and nPP in the diet and further increase in LDH activity when the dietary phytase was supplemented has also been shown by Brenes *et al.*, (2003). The activity of LDH is nonspecific for hepatocellular disease in birds and the enzyme has five isoenzymes, which occur in a wide variety of tissues (Zantop 1997, Thrall 2004). However, the activity of the enzyme in normal bird is reported to be less than 1000 U/L. The authors could not find any clear explanation for the increased activity of the LDH enzyme and further works are needed to elucidate such an effect.

In conclusion, the current study confirms that the addition of phytase to the calcium and non phytate phosphorus deficient diets could improve the overall performance of the broiler chicks, enhance the retention of phosphorus in the body and hence reduce the environmental pollution of phosphorus. However, further studies are needed in order to clarify the influence of phytase on the function of the hepatic enzymes.

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مجله تحقیقات دامپزشکی، ۱۳۸۶، دوره ۶۲، شماره ۴، ۱۱۱-۱۰۳

تأثیر جیره‌های غذایی دارای کمبود کلسیم و فسفر غیر فیتاته به همراه فیتاز بر روی عملکرد رشدی، غلظت سرمی املاح و فعالیت آنزیم‌های آلانین ترانس آمیناز، آسپارتات ترانس آمیناز، لاکتات دهیدروژناز در جوجه های گوشتی

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تحت یک آزمایش تأثیر فیتاز میکروبی (فیز آیم ایکس پی ۵۰۰۰) در جیره غذایی جوجه‌های گوشتی بر روی عملکرد پرنده، پروتئین تام سرمی، املاح و فعالیت آنزیمی سرم (LDH ,AST ,ALT) مطالعه گردید. یک جیره رفرانس کافی از لحاظ کلسیم و فسفر غیر فیتاته (۱۰ گرم در کیلوگرم کلسیم و ۵ گرم در کیلوگرم فسفر غیرفیتاته) و دو جیره دیگر که دارای کمبود کلسیم و فسفر غیرفیتاته بودند (به ترتیب ۸/۵ گرم در کیلوگرم کلسیم و ۳/۵ گرم در کیلوگرم فسفر و ۷/۵ گرم در کیلوگرم کلسیم و ۲/۵ گرم در کیلوگرم فسفر) بدون و با آنزیم فیتاز تهیه و در اختیار جوجه های مورد آزمایش از سن یک روزه تا ۲۱ روزه قرار گرفت. اگرچه جیره‌های دارای کمبود فسفر غیر فیتاته تأثیر معنی‌داری بر روی افزایش وزن بدن جوجه‌ها نداشتند ($p>0.05$) این جیره‌ها باعث افزایش میزان مصرف خوراک و افزایش ضریب تبدیل غذا ($p<0.01$) در مقایسه با گروه‌های مشابه به همراه آنزیم فیتاز گردیدند. فیتاز تأثیر مثبت، اگرچه غیر معنی‌دار، بر روی افزایش وزن بدن جوجه‌هایی که جیره‌های دارای کمبود شدید فسفر غیر فیتاته بودند، داشت. آنزیم باعث کاهش مصرف خوراک ($p<0.05$) و بهبودی ضریب تبدیل غذایی جوجه‌های دریافت کننده جیره دارای کمبود فسفر غیر فیتاته گردید ($p<0.01$) کاهش فسفر غیر فیتاته جیره، باعث افزایش کلسیم سرمی ($p<0.05$) و کاهش فسفر سرمی ($p<0.05$) گردید ولی تأثیری بر روی منیزیوم سرمی نداشت ($p>0.05$). فیتاز جیره باعث کاهش سطح کلسیم سرم و افزایش سطح فسفر سرم در جوجه هایی که جیره دارای کمبود فسفر غیر فیتاته بود گردید ($p<0.05$). فعالیت آنزیم LDH تحت تأثیر جیره دارای کمبود کلسیم و فسفر غیر فیتاته افزایش پیدا نمود ($p<0.01$) در حالی که تأثیری در میزان فعالیت AST,ALT و همچنین مقدار پروتئین تام سرمی مشاهده نگردید ($p<0.05$). فیتاز باعث کاهش فعالیت ALT سرمی ($p<0.05$) شد ولی تأثیری بر روی AST نداشت، فعالیت سرمی آنزیم LDH تحت تأثیر فیتاز افزایش بیشتری پیدا نمود ($p<0.01$).

واژه‌های کلیدی: فیتات فوسفور، فیزیم، برویلر پرفورمانس.

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