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EFFECT OF PHYTASE SUPPLEMENTATION ON THE GROWTH PERFORMANCE OF GROWING RABBITS FED LOW-PHOSPHORUS DIETS.

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ABSTRACT

A total of forty eight New Zealand White rabbits (NZW)of 30 days of age were used to study the growth performance, carcass characteristics, digestibility of nutrients and tibia bone traits of growing rabbits fed low – phosphorus diet (low-P) with or without phytase supplementation. Rabbits were divided into four experimental group, each containing 12 rabbits (4 replicates of each group) to receive one of the four experimental diets which controlled with adequate phosphorus, low phosphorus diet, low phosphorus diet + 750 U phytase/kg diet and low phosphorus diet + 1250 U phytase/ kg diet. The feeding period was extended for 56 days. The results showed that rabbits fed low-phosphorus diet recorded lower values ($P<0.05$) of live body weight, weight gain, and depressed feed conversion ratio compared to control group. Dietary phytase supplementation at 750 and 1250 U/Kg improved significantly ($P<0.05$) the weight gain, feed conversion ratio, growth rate and performance index than rabbits fed phytase free low-P diet. The dressing percentage and digestibility coefficients were significantly ($P<0.05$) increased by phytase supplementation. Tibia bone breaking strength; ash and phosphorus percent improved ($P<0.05$) by phytase supplementation. The rabbits fed low-P diet supplemented with 1250 U phytase/Kg diet showed the highest growth performance, dressing percentage, nutrients digestibility and characteristics of tibia bone than those fed control diet containing adequate phosphorus but without phytase supplementation.

In conclusion, Supplementing deficiency phosphorus diets by 1250 U phytase / kg diets improve the growth performance of growing rabbits.

Keywords: Rabbit, low-phosphorus, phytase, growth performance, carcass, digestibility, tibia bone.

INTRODUCTION

Recently an interest appears in minimizing phosphorus (P) in the ruminant and non-ruminant diets to reduce nutrient excretion and feed costs (Shaw *et al.* 2006). As it is known, about 60 to 80% of the P in cereal grains and oil seeds is bound in the form of phytic acid or phytate (Lott *et al.* , 2000 , Veum *et al.* 2006) which consists of an inositol ring binding up to six phosphate groups that are unavailable for absorption (Maga, 1982).

Phytate is known to complex with other nutrients. Thus, the unavailable phytate P and nutrients complexes with it cannot be utilized and are excreted (Kornegay 2001). Therefore, removing two – thirds or more of dietary inorganic P is sufficient to maintain growth performance and carcass (O'Quinn *et al.* 1997, Mavromichalis *et al.* 1999, Shaw *et al..*, 2002).

Rabbits are able to utilize the phytic P in sunflower meal by 48% (Blanco and Gueguen, 1975) where the apparent digestibility of P was 20% of intake rabbits fed plant P (Leeuwen and Riel, 1978).

Cheeke *et al.* (1985) observed an apparent digestibility values for phosphorus of 35% for red clover meal and 17% for alfalfa meal. They indicated that phosphorus in corn – soybean meal diets for rabbits had an apparent availability of about 50%.

Phytase a member of phosphoesterases group can cleave phosphate group from the phytate molecule. (Lassen *et al.*, 2001, Augesburg *et al.*, 2003. Stahl *et al.*, 2004). It has been shown in literature that supplemental microbial phytase to diets of pigs and poultry improved the utilization of phosphorus and other minerals. Further studies proved that the addition of phytase increased the growth rate and feed conversion ratio. However, studies related to phytase were mainly focused on the efficiency of microbial phytase in diets of pig, broiler, turkey, laying hens, ducks; fish etc, while the efficiency of phytase in rabbits is not observed compared to the animals previously mentioned. It has been reported that phytase exited in intestinal striated brooder of rabbits but its activity is week (Zhao *et al.*, 2004).

Ayers *et al.* (1992) concluded that adding phytase to diet contain 50% roughage and contain low P caused an increase in nutrient digestibility of growing rabbits. El Adawy (2004) and El Adawy *et al.*, (2004) reported that phytase can play an important role to improve the rabbit utilization of diet rich in phytic acid. In this connection, Zhao *et al.*, (2004) concluded that supplemented 800 U/Kg phytase is optimal for rex-rabbits based on the growth performance and economical efficiency. Thus, the aim of this study is to assess the effect of microbial phytase on growth performance, nutrients digestibility, carcass characteristics and mechanical and chemical traits of tibia bone in growing NZ white rabbits fed low phosphorus diet (Low-P diet) with no supplemental inorganic P.

MATERIALS AND METHODS

The experimental work of the present study was carried out at the Rabbits Production Units (RPU), Department of Poultry Production, Faculty of Agriculture, Ain Shams University, Cairo-Egypt.

Rabbits:

A total of Forty eight (48) weanlings male NZW rabbits of, 5 weeks old, where marked immediately after weaning then randomly assigned to four experimental groups, with 12 rabbits treatment, divided into four replicates (3 rabbits/ replicate).

Diets:

Four experimental diets were formulated. The first diet represents the positive control (contain the recommended level of phosphorus using dicalcium phosphate as the main source of P). The second diet is the negative control (consist as the first one but without dicalcium phosphate and contains low-p level). The third and the fourth additional treatments were identical to the low-p diet (negative control) except either 750 or 1250 U phytase was added per kilogram of diet. All four experimental diets are isocaloric, isonitrogenous and isofibrous and formulated to meet all essential nutrient requirements for growing rabbits according to NRC, 1977. Calcium and phosphorus was calculated according to Lebas, 1998. Ingredients composition and chemical analysis of the experimental diets are presented in Table (1).

Phytase activity is expressed as FYT, FTU, PU and U. All are same and one unit of phytase is defined as the amount of enzyme that liberated 1 micromole of inorganic phosphorus per minute from 1.5 mille mole sodium phytate solution at 37°C and pH 5.5.

Table (1): Composition and chemical analysis of the experimental diets:

Ingredient %	Diet (1)	Diet (2)*
Clover hay	37	37
Wheat bran	10	10
Soy bean meal (48%)	16	16
Corn yellow	18.7	18.7
Barley	14	14.6
Limestone	0.3	0.9
Molasses	2	2
Salt (NaCl)	0.5	0.5
Vitamins mixture**	0.3	0.3
Dicalcium phosphate	1.2	0
Total	100	100
Calculated analysis		
DE (Kcal/kg)	2582	2601
Crude protein %	16.27	16.33
Crude fiber %	13.28	13.32
Ether extract %	2.7	2.8
P%	0.65	0.40
Ca%	0.96	0.93
Chemical analysis %		
Crude protein	16.1	15.96
Crude fiber	13.4	13.2
Ether extract	2.2	2.2
NFE	52.2	52.1
P	0.63	0.38
Ca	0.9	0.9

* Phytase supplied per kg of low phosphorus diet at 0, 750 or 1250 unit.

** Each 3 kg. contains: Vit.A 12000000 IU; Vit.D_3 2000000IU; Vit.E 10000 mg; Vit.K_3 2000 mg; Vit.$B_1$1000 mg; Vit.B_2 5000 mg; Vit.B_6 1500 mg; Vit.B_{12} 10 mg; Biotin 50 mg; Coline chloride 250000 mg; Pantothenic acid 10000 mg; Nicotinic acid 30000 mg; Folic acid 1000 mg; Manganese 60000 mg; Zinc 50000 mg; Iron 30000 mg; Copper 10000 mg; Iodine 1000 mg; Selenium 100 mg; Cobalt 100mg and $CaCO_3$ to 3000 g.

Management:

The experimental rabbits were housed in galvanized metal wire cages. Each cage was 60 x 50 x 40 cm for length, width and height, respectively. They provided with feeders and automatic watering system, with three rabbits per each cage. The cages were located in a naturally ventilated and lighting building. The experimental diets in pellet form were offered *ad libitum* and fresh water was available all the time during the experimental period. Rabbits were individually weighed at the beginning of the experiment, then at weekly intervals until the end of the experiment. Daily weight gain, daily feed consumption, feed conversion ratio and mortality rate were recorded.

Growth rate (GR) was calculated as:

FBW-IBW/o.5 (FBW+IBW) x 100

Where,

FBW = Final body weight, IBW = Initial body weight

The performance index (PI) was calculated according to (North, 1981) as follows:

PI= (Live body weight (Kg)/ feed conversion) x 100

The Production Efficiency Factor (PEF) was calculated according to (Emmert, 2000) as follows:

PEF = {Livability (%) x mass (Kg) / FCR x Age in day} x 100

Where:

Livability = 100-Mortality rate (%)

Mass (Kg) = Final live body weight (Kg)

The feeding trial continued for 8 weeks.

Digestibility Trial:

At the last week of the experiment (13 weeks of age), a digestibility trial was conducted using 16 rabbits (four rabbits from each treatment group), which were housed individually in metabolism cages that allow feces and urine separation. The preliminary period continued for 7 days and the collection period extended for 5 days. Feed intake was exactly determined. Feces were collected daily, weighed and dried at 60-70°C for 24 hours, bulked, finely ground and stored for chemical analysis. The apparent digestibility coefficients of DM, OM, CP, CF, EE and NFE for the experimental diets were estimated.

Carcass characteristics:

At the end of the growth trial, six randomly chosen rabbits (13 weeks of age) representing each group were fasted for approximately 16 hrs, individually weighed (to record the pre-slaughter weight) and therefore slaughtered according to the standard technique of Cheeke (1987). Dressing percentage included relative weights of carcass (without head) and giblets (liver, heart and kidney) were calculated.

Bone parameters:

From the same rabbits of the slaughter test; tibia was removed from the left leg, cleaned from flesh and all soft tissues. Tibia length (TL) cm, width (TW) and tibia circumference (TCF) were measured using a caliper as described by Samejima, (1990). Tibia breaking strength (TBS) was measured using a universal testing machine, (Tinuls Olsen Toting Machine Co.), in Materials department, Faculty of Engineering - Ain Shams University). Tibia bones were oven dried at 80°C for 24h, and then tibia were ashed in muffle furnace at 600°C for 6h to conduct the chemical analysis according to the methods of Association Official Analytical Chemists (A.O.A.C., 1990). Tibia ash (TA) was expressed as a percentage of the dry weight.

Chemical analysis:

Proximate analysis of the experimental diets and feces for moisture, CP, EE, Ash and NFE were carried out according to A.O.A.C. (1990).

Statistical analysis:

Data were analyzed statistically using one-way analysis of variance methods according to SAS (1995). Duncan's mew multiple range procedure was followed to separate means (Duncan's, 1955)

The model applied was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = The observation on the Ith treatment.

μ = Overall mean.

T_i = Effect of the Ith treatment.

e_{ij} = Random error treatment.

RESULTS AND DISCUSSION

Growth performance and feed utilization:

Growth performance data of growing NZW rabbits as affected by the experimental diets are presented in Table (2). Results indicated that, Means of live body weight and body weight gain from 5 to 13 weeks of age were improved (P<0.05) by phytase addition. The magnitude of the response was greater for rabbits fed low- P diet supplemented with 1250 and 750 units of phytase. The lowest body weight was recorded (P<0.05) for rabbits received diet containing low P level. No effect was noted for average feed intake during the experimental period (5-13 weeks of age).

Feed conversion ratio tended to be improved significantly (P<0.05) compared with the negative control diets (rabbits fed on low-P diets without phytase supplementation) by 0.9 % and 0.92%, respectively. These tendencies may indicate some effect of phytase on performance beyond the deficiency phosphorus related effect (kies *et al.* 2001).

Adding phytase to Low-P basal diet increased the growth rate performance index and the production efficiency factor than rabbit fed phytase free low-P diet and to levels equal to or exceeding than those fed control diet.

A number of studies have demonstrated that addition of moderate proportional microbial phytase to low phosphorus diets significantly improved growth performance of weanling pigs (Shaw *et al.*, 2006), broiler chicks, laying hens (Ravandran, 2000; Xu *et al.*, 1997, Ghazalah *et al.*, 2006), and ducks (Wang *et al.*, 2000). The above results agree with El Adawy (2004) who reported that the supplemental phytase at 500 and 1000 FTU / Kg improved (P<0.05) the weight gain, feed conversion and performance index. Such findings are confirmed by (Zhao *et al.*, 2004) who concluded that in comparison with negative control (without dicalcium phosphate), the average daily gain increased by 1.65%, 5.77%, and 11.48% for rabbit fed diet supplemented with 400, 600, 800 U/KG diet, respectively.

The improvements in growth performance observed in growing rabbits fed phytase may be due to:

1. Releasing of minerals from the phytate mineral complex (Simons *et al.* 1990 and Schoner *et al..*, 1991).
2. Increasing the availability of protein utilization (Ravindran *et al.*; 1999).
3. improving energy utilization of rabbit diets (Ravindran *et al..* ;2000)

It is clear that phytase supplementation overcome the depression of growth rate observed for rabbits fed low-P diet.

Table (2): Effect of phytase supplementation to the low-P diets on the growth performance of growing rabbits.

Items	Control	Low-P diet	Low-P +750 Units phy.	LowP+1250 Units phy.	Sig.
Initial body weight (g)	554±22.1	556±18.2	568±24.2	555±34.4	NS
Final body weight (g)	1964^{b}±44.2	1918^{c}±43.1	1940^{b}±52.4	2026^{a}±42.6	*
Total weight gain (g)	1409^{b}±34.1	1362^{c}±28.2	1371^{b}±44.4	1470^{a}±22.4	*
Daily feed intake (g)	93.0 ±7.3	95.4 ±12.4	88.6 ±14.2	93.8 ±14.1	NS
FCR (g feed/ g gain)	3.7^{bc}±0.01	3.9^{c}±0.03	3.6^{b}±0.02	3.5^{a}±0.01	*
Mortality rate %	-	25	-	-	*
Growth rate%	111.9^{b}	107.8^{c}	109.3^{bc}	113.2^{a}	*
PI %	53.08^{b}	47.38^{c}	53.88^{b}	57.23^{a}	*
PEF%	94.87^{b}	63.46^{d}	81.34^{c}	102.19^{a}	*

a, b and c means having the same common letter within each row are not significantly different (P<0.05).

Nutrient digestibilities for the experimental diets:

Digestibility coefficients of the experimental diets are presented in Table (3). Supplementation of phytase to low-P diets improved (P<0.05) DM, OM, CP, EE, CF and NEE digestibility than those fed low-P diets without phytase. Ayers *et al.* (1992) concluded that adding phytase at 1% to ration (50% roughages) fed to rabbits at 0.27% P level caused an increase in DM and CP digestibility. El-Adawy (2004) reported that, digestibility values of DM, OM, CP, CF, EE and NFE in rabbits fed diets supplemented with 1000 FTU/Kg diet were significantly (P<0.01) increased by 6.2, 5.8 , 7.1 , 12.3 , 4.3 and 6.1% respectively than those fed diets without phytase supplementations.

Table (3): Effect of phytase supplementation to the low-P diets on nutrients digestibility of growing rabbits.

Items %	Control	Low-P diet	Low-P+750 units phy.	Low-P+1250 units phy.	Sig
DM	71.66^{b} ±4.0	66.82^{c} ±4.5	70.56^{b} ±3.3	73.2^{a} ±8.0	*
OM	74.77^{b} ±2.9	71.59^{c} ±4.2	74.6^{b} ±2.3	77.4^{a} ±5.8	*
CP	70.0^{a} ±3.9	63.8^{b} ±6.3	68.3^{a} ±8.9	70.9^{a} ±4.4	*
EE	79.68^{ab} ±3.8	69.7^{c} ±5.5	83.1^{a} ±5.2	83.2^{a} ±6.4	*
CF	33.3^{b} ±12.3	28.0^{c} ±6.1	32.1^{b} ±4.2	36.1^{a} ±2.2	*
NFE	81.4^{a} ±1.5	76.0^{b} ±2.2	81.2^{a} ±3.0	80.8^{a} ±2.4	*

a, b and c means having the same common letter within each row are not significantly different (P<0.05)

Carcass Characteristics:

Dressing percentage was significantly increased with the dietary phytase supplementation (Table 4). Dressing percentage of rabbits fed low-P diets supplemented with phytase improved significantly ($P<0.05$) than those fed low-P diet.

Increasing the dietary phytase supplementation did not have any significant effect on liver and kidneys percentage. These results confirmed the previous studies reported by El-Adawy *et al.* (2004) who observed significant ($P<0.05$) increase in dressing percentage of rabbits fed phytase supplemented diets.

Table (4): Effect of phytase supplementation to the low-P diets on carcass characteristics of growing rabbits.

Items	Control	Low-P diet	LowP+750 Unit phy.	LowP+1250 it phy.	Sig.
Live body weight, g	1980±24.1	1924±32.0	1938±24.4	2040±18.1	NS
Dressing %	60^{a} ±0.7	56.5^{c} ±0.8	58.5^{ab}±0.9	61.8^{a} ±1.2	*
Liver weight %	3.1 ±0.11	2.9 ±0.13	3.06 ±0.17	3.08 ±0.12	NS
Kidneys weight %	0.61 ±0.06	59 ±0.05	0.62 ±0.02	0.62 ±0.05	NS
Heart %	0.32 ±0.03	0.30 ±0.05	0.30±0.04	0.31 ±0.03	NS

a, b and c means having the same common letter within each row are not significantly different ($P<0.05$).

Effect of phytase supplementation to the low-P diets on Tibia bone characteristics:

The total percentage of tibia bone strength increased significantly ($P<0.05$), with phytase supplementation to low-P diets. The tibia of rabbits fed low-P diet supplemental with 750 or 1250 units of phytase were numerically longer and have more circumference than those fed the P-deficient diets (Table 5). At the same time, supplementing the diet with phytase markedly improve tibia breaking strength. In this respect, Qian *et al.* (1996) reported that the mechanical traits (weight, length, tibia circumference and breaking strength) were increased when phytase was added to low-P diet. Rabbits fed low P diets supplemented with 750 or 1250 units of phytase showed a significant ($P<0.5$) improvement in the percentage level of tibia ash and P than those fed low-P diets (Table 6). But, tibia calcium (Ca) was not improved by phytase supplementation. Zhao, *et al.*, (2004) reported that phytase supplementation to Rex-rabbit diet can improve the phytase phosphorus utilization by increasing efficiency. Moreover, Broz *et al.* (1994) reported that phytase supplementation to corn- soybean diets increased the tibia ash percentage in chickens. Perney *et al.* (1993) concluded that the improvement in percentage tibia is a good indication of increased bone mineralization due to the fact that there is an increased availability of P, Ca and Zn from the phytic mineral complex by the action of phytase. Also Rowland *et al.*, (1997) reported that percentage of bone ash is usually positively correlated with bone breaking strength. It may be suggested that stronger bones of growing rabbits fed diets containing supplemental phytase resulted from the better mineralization of bone.

The results of tibia bone lead to suggest that supplemental phytase increases the availability of P and promotes the growth and development of the bone, thus the amount of inorganic P added to the diet could be reduced.
It could be concluded that reformulating low-phosphorus rabbit diet with 1250 U phytase/ kg diet could improve growth performance and feed conversion rather than dressing percentage of growing rabbits form 30-86 days of age.

Table (5): The effect of phytase supplementation to the low P-diets on the chemical traits of tibia bone in growing rabbits.

Items	Control	Low-P diet	Low-P+750 unit phy.	LowP+1250 unit phy.	Sig
Tibia Breaking Strength (kg/cm^3)	10.58^b±0.8	8.0^c±0.6	11.3^b±0.48	15.5^a±0.52	*
Tibia Length (cm)	9.2±0.18	9.0±0.16	9.1±0.16	9.4±0.14	NS
Tibia Width (cm)	0.65±0.02	0.63±0.01	0.63±0.01	0.7±0.02	NS
Tibia Circumferences (cm)	2.05 ± 0.08	1.93±0.07	2.1±0.04	2.15±0.02	NS

a, b and c means having the same common letter within each row are not significantly different.

Table (6): The effect of phytase supplementation to the low-P diets on the chemical traits of Tibia bone in growing rabbits

Items %	Control	Low-P	Low-P+750 units phy.	LowP+1250 units phy.	Sig
Ash	48.2 ± 0.13	44.1 ± 0.37	47.2 ± 0.47	49.6 ± 0.4	*
P	16.2±0.14	13.4±0.16	15.5±0.12	17.1±0.22	*
Ca	37.4±0.20	36.4±0.16	36.8±0.20	37.6±0.26	NS

a, b and c means having the same common letter within each row are not significantly different (P<0.05).

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تأثير إضافة انزيم الفيتيز على مظاهر النمو في الأرنب المغذاه على علائق منخفضة في الفوسفور

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اجريت هذه التجربة بهدف دراسة تأثير اضافة انزيم الفيتيز الى علائق منخفضة في محتواها من الفوسفور على مظاهر النمو وصفات الذبيحة ومعاملات الهضم وصفات عظمة الساق في الارانب النامية.

وقد تم استخدام عدد ٤٨ ارنب نيوزلندي أبيض في عمر ٣٠ يوم موزعة على اربع مجاميع تجريبية كل منها تحتوي على ١٢ أرنب (٤ مكررات لكل مجموعة).

تم تركيب علائق المقارنة بحسث تحتوي العليقة الأولى على الاحتياجات المطلوبة من الفوسفور وعليقة المقارنة السالبة تحتوي على مستوى منخفض من الفوسفور يضاف اليها انزيم الفيتيز بمتسوى صفر ، ٧٥٠ ، ١٢٥٠ وحدة فيتيز لكل كجم علف.

وكانت أهم النتائج المتحصل عليها كالتالي:

- الأرانب المغذاه على علائق منخفضة في محتواها من الفوسفور انخفض فيها معنويا الوزن الحي ومعدلات الزيادة الوزنية علاوة على تدني معامل التحويل الغذائي ودليل الكفاءة مقارنة بالارانب المغذاه على عليقة المقارنة.
- اضافة انزيم الفيتيز بمعدلات ٧٥٠ و ١٢٥٠ وحدة فيتيز / كجم عليقة الى العلائق المنخفظة في محتواها من الفوسفور ادى الى تحسن معنوي في الوزن الحي ومعدلات الوزن المكتسب وتحسن معامل التحويل الغذائي ودليل الكفاءة.
- اضافة انزيم الفيتيز ادى الى تحسن في نسبة التصافي ومعدلات الهضم.
- تحسنت مواصفات عظمة الساق (قوة الكسر، النسبة المئوية للرماد ونسبة ترسب الفوسفور) في الارانب المغذاه على علائق مضاف اليها انزيم الفيتيز.
- اظهرت الارانب المغذاه على عليقة منخفضة في محتويات الفوسفور مضاف اليها ١٢٥٠ وحدة فيتيز /كجم تفوق معنوي في الوزن الحي ومعدلات الزيادة الوزنية ومعامل التحويل الغذائي ونسبة التصافي ومعاملات الهضم علاوة على تحسن قوة الكسر بالنسبة لعظمة الساق ونسبة الرماد ونسبة ترسب الفوسفور مقارنة بالمجموعة المغذاه على عليقة المقارنة والمحتواه على الكمية المطلوبة من الفوسفور ولكن بدون اضافة انزيم الفيتيز

وعموما فان اضافة نزيم الفيتيز بمستوي ١٢٥٠ وحدة / كجم علف الى العلائق المنخفضة في محتواها من الفوسفور يؤدي الى تحسين صفات النمو والذبيحة ومعاملات الهضم وصفات عظمة الساق في الارانب النامية.