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EFFECT OF PHYTASE LEVELS ON PERFORMANCE OF GROWING RABBITS UNDER EGYPTIAN CONDITIONS

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ABSTRACT: This study aimed to examine the effects of food supplementation with varying amounts of phytase on growth performance, nutrient digestibility, and blood parameters of growing rabbits in summer settings in Egypt. An experimental design was conducted incorporating three levels of phytase: 0.0, 1.5, and 3.0 g/kg of food, along with a control group, during the winter season. Results demonstrated that growth performance, daily feed intake, feed conversion ratio, nutritional digestibility, nutritive values, hemoglobin, hematocrit, alanine aminotransferase (ALT), and red blood cell counts were considerably diminished throughout the summer season in comparison to the winter control. Conversely, phytase levels significantly enhanced growth performance indicators, crude fiber (CF) and ether extract (EE) digestibility, as well as other blood parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, triglycerides, and cholesterol in growing rabbits. In conclusion, the findings indicated that the fortification of rabbit diets with phytase, particularly at levels of 1.5 and 3.0 g/kg, could improve the growth performance of rabbits during mild and hot climates in Egypt.

Key words: Rabbits, phytase, growth performance, digestibility, blood biochemistry.

INTRODUCTION

In Egypt, recent years have witnessed an increasing interest in commercial rabbit production because to their elevated reproductive rates, swift growth rates, compact body size, and substantial meat yield (**Youssef *et al.*, 2008**). However, increasing temperatures remain a significant obstacle in the rabbit industry due to their detrimental effects on feed consumption, weight increase, feed efficiency, meat quality, mortality rates, and overall health of rabbits (**Marai *et al.*, 2008; Hassan *et al.*, 2016**).

Moreover, changes in rectal, skin, and ear temperatures, respiration rate, thyroid and stress hormones, as well as levels of albumin, globulin, total lipids, glucose, sodium, potassium, calcium, magnesium, and phosphorus are the primary physiological responses to heat load in rabbits (**Marai *et al.*, 2008**). Diverse genetic, managerial, dietary, buffering, hormonal, and physical techniques have been implemented to alleviate the deleterious consequences of heat stress (Fa**yez *et al.*, 1994; Marai *et al.*, 1999**).

Phytase is an enzyme that enhances the digestion of phytate phosphorus, hence improving the total availability of dietary phosphorus. The incorporation of this enzyme as a feed addition in swine diets diminishes the necessity for inorganic phosphorus supplementation. Replacing inorganic phosphorus with corn or other grains in the diet formulation enhances the overall energy content of the diet. Enhanced phosphorus consumption and diminished inorganic phosphorus in the diet lead to decreased phosphorus excretion from swine. Phytase can

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also enhance the bioavailability of other minerals, including calcium. Due to phytase's sensitivity to elevated temperature and humidity, appropriate storage and handling protocols must be adhered to in order to preserve the product's efficacy. The thermal stability of the product must be taken into account when feeds are pelleted. **(Dersjant-Li *et al.*, 2015)**.

MATERIALS AND METHODS

This study was conducted at the Rabbit Research Farm and Laboratories inside the Animal Production Department, Faculty of Agriculture, Zagazig University, Egypt.

In the initial experiment, the rabbits were randomly allocated into four equal groups, with ten rabbits each group. During the winter (mild conditions), the initial group was raised and provided with the control food devoid of phytase addition. The remaining three groups were raised during the summer season (a thermal stress condition) and provided with a control diet augmented with phytase at doses of 0.0, 1.5, and 3.0 g/kg of diet. These quantities were blended with one kilogram of feed and incrementally incorporated into the remaining diet to achieve a uniform supplement concentration. The experiment lasted for eight weeks. The initial trial results indicate that a phytase dosage of 3.0 g/kg yielded the greatest enhancement in rabbit performance.

Forty weaned male developing New Zealand White rabbits (average beginning weight, 714.00 ± 39.57g) were randomly assigned to four food treatment groups. The control group received a baseline diet, whilst the other three groups were provided diets fortified with phytase at concentrations of 0.0, 1.5, and 3.0 g/kg, respectively. Feed and water were provided ad libitum. The experimental duration was prolonged by 8 weeks (from 5 to 13 weeks of age). The rabbits were individually kept in galvanized wire cages of 35 × 60 × 35 cm, equipped with food and automatic drinkers. All groups were maintained under identical management and hygienic circumstances. The live body weight (LBW) of rabbits was documented weekly in grams, and the average daily weight gain (DWG) was computed for each individual. The average daily feed intake (DFI) was documented weekly, and the feed conversion ratio (FCR), expressed as grams of feed per gram of gain, was computed. The mortality rate was documented on a weekly basis.

During the experiment period, ambient temperatures and relative humidity in the rabbitry were recorded using an automatic thermo-hygrometer (OC 14:140, H 10 – 99%; TFA Dostmann GmbH + Co. KG, Wertheim, Germany) twice daily at 08:30 and 14:30 hours. The temperature humidity index (THI) was computed utilizing the modified equation by **Marai *et al.* (2000)** as the following equation:

$$THI = db\,^{\circ}C - [(0.31 - 0.31\,RH)(db\,^{\circ}C - 14.4)].$$

Where, db°C represents the dry bulb temperature in degrees Fahrenheit, and RH denotes the relative humidity expressed as a percentage. The acquired THI values were subsequently classified as follows: 27.8°C indicates the absence of heat stress; 27.8–28.9°C signifies moderate heat stress; 28.9–30°C denotes severe heat stress; and temperatures beyond 30°C represent very severe heat stress.

At the ending of the experimental period, the apparent nutritional digestibility of the experimental diets was assessed. Four rats from each experimental group were individually housed in metabolic cages designed for feces separation. The feed intake was precisely measured. Excreted feces were collected in labeled polyethylene bags, and samples were obtained for chemical analysis. Proximate studies of the experimental meals and fecal samples were conducted. Digestible energy (DE) was computed using the equation of **Schieman *et al.* (1972)**. The total digestible nutrients (TDN) was calculated.

At the end of the feeding period, blood samples were obtained from four rabbits at the time of slaughter to assess blood metabolites. The erythrocyte (RBCs), total leukocyte (WBCs), hemoglobin (Hb) concentration, hematocrit, and lymphocyte counts were assessed using the specified methodology **Grindem (2011)**. Additionally, serum total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, urea, creatinine, and total triglycerides were assessed

utilizing commercial kits acquired from Diamond Diagnostics Company, Egypt. The globulin values were derived by deducting the albumin values from the total protein values. Additionally, the carcass and internal organs (liver, kidneys, heart, lungs, spleen, and caecum) were excised from the corpse and subsequently weighed. Economic evaluation was calculated according to **Ayyat (1991)**.

The data were statistically analyzed on a The differences among the investigated groups were analyzed with a One-way ANOVA test by the following model:

$$Yij = \mu + Ti + eij$$

Where, μ= the overall mean, Ti = the fixed effect of treatment, eij = residual error. The Duncan's new multiple range test (**Duncan, 1955**) was adopted to compare significant differences among means at $P < 0.05$.

RESULTS AND DISCUSSION

Temperature Humidity Index

The mean ambient temperature, relative humidity, and temperature humidity index (THI) within the rabbitry were 20.26°C, 67.41%, and 21.41 in winter, and 29.34°C, 45.46% and 31.44 in summer, respectively, indicating no heat stress in winter and significant heat stress in summer. These results were similar to those of **Maria *et al.* (2000) and Abd El-Moneim *et al.* (2016)** under the same Egyptian climate condition.

Growth Performance and Feed Utilization

Data in Table 2 presented that phytase supplementation had significant (P<0.01) effect on LBW at all experimental intervals. LBW improved in rabbit groups fed diet supplemented with 1.5, or 3.0 g phytase /kg diet, respectively at 6 and 8th week of the experimental period, when compared with those fed the diets without any supplementation. Dietary phytase supplementation in growing rabbit diets was significantly (P<0.001) affected on DBWG (Table 2). At 6th and8th weeks of the experimental period, DBWG increased on rabbits fed diet supplemented with 1.5, or 3.0 g phytase/kg diet, when compared with control group. These results coincide with those obtained by **Singh *et al.* (2003)** who reported that phytase supplementation to broiler diets caused numerical improvement in feed efficiency of broilers.

The enhancement in growth performance noted in rabbits administered phytase was linked to improved feed efficiency, potentially resulting from the liberation and utilization of phosphorus from the phytate-mineral complex **(Qian *et al.*, 1996; Sebastain *et al.*, 1996**) or utilization of inositol (Simons et al., 1990) or increase starch digestibility (**Knuckles and Betschart, 1987**) or increased utilization of protein and amino acids (**Ravindran *et al.*, 2000**) or overall utilization of nutrients **(Miles and Nelson, 1974**).

Presence of dietary phytase was increased significantly affected the DFI(daily feed intake) of growing rabbit during all periods of the experimental study (Table 3). FCR (feed conversion ratio) was significantly (P<0.01 and 0.001) enhanced as a result of phytase supplementation at all weeks of the experimental period compared with those fed control diet. Overall the period, FCR was enhanced for rabbits fed diet supplemented with 1.5 or 3.0 g phytase /kg, when compared with control group (Table 3). These results agree with those obtained by **Ravindan *et al.* (2001), Singh *et al.* (2003), Bozkurt *et al.* (2006) and Mondal *et al.* (2007)** who observed improved feed conversion ratio due to phytase supplementation. The enhancement in feed conversion might be due to the release and utilization of P from the phytate-mineral complex or utilization of inositol, increase starch digestibility or increased utilization of protein and amino acids, or overall utilization of nutrients (**Miles and Nelson, 1974; Knuckles and Betschart, 1987; Simons *et al.*, 1990; Qian *et al.*, 1996; Sebastain *et al.*, 1996; Ravindran *et al.*, 2000**).

Digestibility Coefficients and Nutritive Values

Results in Table 4 didn't show any significant effect on nutrient digestibility due to different dietary levels of phytase, except crude fiber and ether extract which was significantly increased as a result of dietary 1.0and3.0 g phytase/kg diet Similarly, **Marai *et al.* (2004)** observed the reduction in the digestibility coefficients of crude protein and crude fiber of 8.1% and 1.0%,

Table 1. Formulation and chemical analyses of the basal-diets fed to rabbits

Ingredient (%)	**(%)**
Alfalfa hay	29
Yellow corn	23
Wheat straw	4
Wheat bran	29
Soybean meal	13
Sodium chloride	0.5
Limestone	1.2
Minerals and vitamins mixture*	0.3
Total	100
Chemical analyses (% on DM basis), determined	
Organic matter	90.56
Crude protein	18.53
Crude fiber	12.39
Ether extract	4.87
Nitrogen free extract	54.78

* Each 1.5 kg of minerals and vitamins mixture contains: manganese 80 g, zinc 60 g, iron 30 g, copper 4 g, iodine 0.5 g, selenium 0.1 g and cobalt 0.1 g, vitamin A 12000000 IU, vitamin D_3 3000000 IU, vitamin E 10000 mg, vitamin K_3 2000 mg, vitamin B_1 1000 mg, vitamin B_2 5000 mg, vitamin B_6 1500 mg, vitamin B_{12} 10 mg, Biotin 75 mg, folic acid 1000 mg, nicotinic 30000 mg and pantothenic acid 10000 mg.

Table 2. Growth performance of New Zealand White rabbits as affected by phytase leves

	Initial	**2nd week**	**4th week**	**6th week**	**8th week**
Live body weight (g) at					
Control winter	714.00±39.57	1040.83±35.66^{a}	1425.83±22.15^{a}	1833.17±33.86^{a}	2164.17±21.27^{a}
0.0 g phytase/ kg diet	698.33±14.66	937.17±14.86^{b}	1227.17±33.22^{c}	1554.17±39.38^{d}	1881.67±45.89^{b}
1.5 gphytase/ kg diet	690.22±22.07	956.33±22.59^{b}	1289.00±19.86^{bc}	1642.89±19.60^{c}	1969.44±28.39^{b}
3.0 gphytase/ kg diet	704.33±21.58	1011.00±22.41^{ab}	1361.11±27.49^{ab}	1732.22±29.99^{b}	2077.22±24.01^{a}
Significance	NS	*	***	***	***
Live body gain (g) at					
Control winter	23.34±0.92^{a}	27.50±1.11^{a}	29.10±1.05^{a}	23.64±1.26	25.90±0.56a
0.0 g phytase/ kg diet	17.06±0.57^{b}	20.71±1.59^{c}	23.36±1.09^{b}	23.39±0.99	21.13±0.67c
1.5 gphytase/ kg diet	19.01±0.84^{b}	23.76±0.93^{b}	25.28±0.69^{b}	23.33±1.24	22.84±0.40b
3.0 gphytase/ kg diet	21.90±0.80^{a}	25.01±0.62^{ab}	26.51±1.29^{ab}	24.64±1.11	24.52±0.38a
Significance	***	***	**	NS	***

Means in the same column bearing different letters differ significantly (P<0.05).
NS = Not significant and *P<0.05., ***=p<0.001

Table 3. Daily feed intake and feed conversion ratio New Zealand White e rabbits as affected by phytase levels.

	Initial	2nd week	4th week	6th week	8th week
Daily feed intake (g) at					
control winter	61.62±2.56^{a}	100.38±3.79^{a}	138.12±5.92^{a}	184.73±3.51^{a}	121.21±3.20^{a}
0.0 g/ kg diet	51.55±2.18^{b}	89.57±2.75^{b}	125.73±3.05^{b}	165.61±2.44^{b}	108.11±1.98^{b}
1.5 g/ kg diet	48.67±1.65^{b}	81.80±2.16^{b}	117.82±2.49^{b}	156.96±1.69^{c}	101.31±1.70^{c}
3.0 g/ kg diet	51.15±1.83^{b}	88.28±.2.58^{b}	124.18±1.95^{b}	161.44±2.61^{bc}	106.27±1.79^{bc}
Significance	**	**	**	***	***
feed conversion ratio					
control	2.66±0.16^{ab}	3.71±0.29^{b}	4.75±0.11^{b}	8.06±0.74	4.70±0.20^{b}
0.0 g/ kg diet	3.03±0.12^{a}	4.44±0.33^{a}	5.42±0.15^{a}	7.15±0.32	5.13±0.11^{a}
1.5 g/ kg diet	2.59±0.13^{b}	3.47±0.11^{b}	4.68±0.12^{b}	6.89±0.39	4.44±0.07^{b}
3.0 g/ kg diet	2.37±0.14^{b}	3.54±0.12^{b}	4.79±0.27^{b}	6.66±0.32	4.35±0.11^{b}
Significance	*	*	*	NS	**

Means in the same column bearing different letters differ significantly (P<0.05).
NS = Not significant and *P<0.05., ***=p<0.001

Table 4. Digestibility and nutritive value of the experimental diets as affected by season, phytase level or their interaction

	Digestibility coefficient (%)					Nutritive values (%)			
	DM	OM	CP	CF	NFE	EE	DCP	TDN	DE
Phytase level effect									
Control winter	53.42±1.28	83.62±0.22	69.89±0.64	54.52±1.61^{a}	67.51±0.77	62.19±0.96^{a}	13.35±0.12	70.19±0.87	3094.33±38.11
0.0 g/ kg diet	64.45±3.00	62.85±2.47	72.40±3.08	31.41±1.19^{b}	73.43±2.34	64.68±2.97^{b}	12.45±0.53	69.27±2.36	3044.33±104.81
1.5 g/ kgdiet	60.26±3.83	58.65±2.01	74.33±2.63	34.06±5.02^{b}	75.83±4.01	67.65±4.16^{b}	12.79±0.45	71.34±3.74	3135.25±162.05
3.0 g/ kg diet	61.07±2.81	58.82±2.75	70.51±2.31	29.13±2.03^{b}	72.30±2.04	62.65±2.57^{b}	12.13±0.40	67.70±1.98	2975.00±87.35
Significance	NS	NS	NS	**	NS	***	NS	NS	NS

Means in the same column bearing different letters differ significantly (P<0.05).
NS = Not significant and *P<0.05., ***=p<0.001

respectively, was reported in New Zealand White rabbits during summer compared to winter. The diminished digestibility of nutrients may occur from a decline in the synthesis of digestive enzymes caused by heat stress **(Habeeb *et al.*, 1992)**. The phytate also binds with the major proteolytic enzyme, trypsin (**Caldwell, 1992**) eventually leading to lowered digestibilties of nitrogen and amino acids. Consequently, it is probable that when phytase hydrolyzes the ester bonds to liberate phosphorus from the phytic acid molecule, it will also release the protein bound to phytate and mitigate the adverse effects of phytic acid on proteolytic enzymes, thereby enhancing the digestion and absorption of protein and amino acids (**Ravindran *et al.*, 2000**).

Blood Parameters

As shown in Tables 5 and 6 white blood cell count and Red blood cells counts were significantly (P<0.05) increased, while cholesterol, creatinine, urea,ALT and AST decreased on level 0.3mg/kg phatase compared with winter group In the same trend, results of serum ALT and AST of the present study are agreed with (El-Badry *et al.*, 2008) who found that ducks fed diet contained phytase recorded highest levels of creatinine, AST and ALT without any change in blood hematology compared to other groups.

The similarity of enzyme activity in supplemented phytase groups demonstrates the beneficial, non-pathological, or non-toxic effects of the evaluated biological additions on liver function. Variations in blood transaminase levels may be contingent upon the rate of protein metabolism, which could be influenced more by the bird's age than by any other factor. It is widely recognized that by the process of transamination, an amino group is transferred to an alpha-keto acid, while the keto oxygen is transferred to the amino group donor, facilitated by transaminases **(Guyton, 1981).**

Yahav *et al.* (1997) and El-Badry *et al.* (2008) reported that during the high temperature phase plasma osmolality was significantly reduced. Also, they added that the increase in concentration of plasma protein could due to phytase supplementation be important in this regulatory response, which also resulted in a decrease in osmolality.

The reduction in blood cholesterol levels may result from a diminished rate of cholesterol absorption via the intestinal villi, leading to lower plasma cholesterol levels. Alternatively, this decrease in total plasma cholesterol may also be attributed to phytase's action on the enzyme 7-alpha hydroxylase, which regulates cholesterol catabolism **(Wahba, 1969).** This reduction may be logically significant, as cholesterol is oxygenated in the liver, resulting in the creation of bile acids that exist as anions. Bile salts secreted into the gut for lipid breakdown **(Stroev, 1989).** In addition, **Wahba (1969)** was revealed that bile salts are significant end products in cholesterol metabolism.

Table 5. Haematological parameters of New Zealand White rabbits as affected by phytase levels

	RBCs count (10^6/ml)	Hemoglobin (g/dl)	Hematocrit (%)	WBCs (10^3/ml)	Lymphocytes (10^3/ml)
Phytase level effect					
Control winter	4.52±0.21^a	11.00±**0.44**	0.32±0.02	8.03±0.09^a	2.91±0.39
0.0 g/ Kg diet	3.56±0.18^b	9.37±0.22	0.25±0.01	5.60±0.57^b	3.71±0.11
1.5 g/ Kg diet	3.81±0.22^b	9.75±0.40	0.28±0.02	5.29±0.741^b	3.46±0.17
3.0 g/ Kg diet	3.66±0.17^b	10.03±0.37	0.26±0.01	564.21±^{b}	3.29±0.26
Significance	*	NS	NS	*	NS

Means in the same column bearing different letters differ significantly (P<0.05).
NS=Not significant, **P<0.01 and *** P<0.001.

Table 6. Blood biochemical parameters of New Zealand White rabbits as affected by phytase levels

	ALT (u/l)	AST (u/l)	Urea (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total Protein (mg/dl)	Albumin (mg/dl)	Globulin (g/d)	Albumin/ Globulin
phytase level effect										
Control winter	45.48±0.96^b	51.10±7.20$_b$	30.44±1.24^a	0.61±0.01	108.49±3.16^a	108.49±1.31^a	6.09±0.09	3.42±0.25	2.67±0.34	1.39±0.29
0.0g/ kgdiet	43.24±4.92^b	43.84±3.63^b	21.29±1.73^b	0.68±0.04	95.44±1.54^b	110.56±4.94^a	5.89±0.25	3.04±0.02	2.85±0.28	1.10±0.12
15. g/ kgdiet	53.616.81±3.61 ^{a}	55.77±2.38^{ab}	30.60±1.59^a	0.62±0.02	89.65±4.48^{bc}	93.89±4.29^b	6.24±0.33	3.63±0.19	2.62±0.49	1.55±0.26
3.0 g/ kgdiet	63.87±**2.14**^{a}	68.11±4.62^a	26.66±2.68^{ab}	0.62±0.02	83.83±2.07^c	102.07±3.93^{ab}	6.43±0.17	3.32±0.09	3.12±0.10	1.21±0.08
Significance	**	*	*	NS	**	*	NS	NS	NS	NS

Means in the same column bearing different letters differ significantly (P<0.05).
NS=Not significant, **P<0.01 and *** P<0.001.

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تأثير إضافة مستويات انزيم الفيتيزعلى أداء النمو فى الأرانب النامية تحت الظـــروف المصرية

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أجريت هذه الدراسة لمعرفة تأثير المكملات الغذائية بمستويات مختلفة من الفيتيز على أداء النمو، وهضم العناصر الغذائية، وصفات الدم للأرانب النامية تحت ظروف الصيف في مصر. تم تنفيذ تجربة تضمنت ثلاثة مستويات من الفيتيز 0.0، 1.5، 3.0 جرام/كجم عليقةفى الصيف ومجموعة الكنترول في الشتاء. أشارت النتائج إلى انخفاض معنوي في أداء النمو، الاستهلاك اليومي من العلف، نسبة التحويل الغذائي، هضم العناصر الغذائية، القيم الغذائية، الهيموجلوبين، الهيماتوكريت، انزيم ALT، عدد خلايا الدم الحمراء في الصيف مقارنة بمعاملة الشتاء. في المقابل، أدت مستويات الفيتيز إلى زيادة معنوية في أداء النمو، وعامل هضم الألياف الخام ومستخلص الإيثر، وبعض قياسات الدم التي تسحب إنزيم ALT، AST، اليوريا، الدهون الثلاثية والكوليسترول) في الأرانب النامية. أوضحت النتائج أن اضافة انزيم الفيتيز فى علائق الأرانب وخاصة المستوى 1.5 و 3.0 جم/كجم عليقة يمكن أن يحسن أداء نمو الأرانب النامية خلال المناخ المعتدل والحار في مصر.

الكلمات الاسترشاديه: الأرانب، إنزيم الفيتيز، اداء النمو، معاملات الهضم،قياسات الد م البيوكيمائة.

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