Effect Of Phytase Enzyme Supplementation On The Performance, Immune Functions And Some Biochemical Parameters Of Broilers

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ABSTRACT

Sixty chicken, one day old commercial Hubbard broiler chicken were equally divided into 3 groups to determine the effect of supplementing a corn-soybean meal based diet with phytase enzyme on growth performance, immune function and some biochemical parameters. The birds were reared under standard hygienic conditions and fed on corn-soybean based balanced commercial ration. Group 1 was fed on a standard ration and kept as control. Groups 2 & 3 were fed on a diet supplemented with 500 and 1000 unit of phytase /kg ration respectively for 6 weeks. Blood samples were collected from each group at the end of the 3rd and 6th week from the start of experiment. The phytase enzyme supplementation significantly increased body weight and improved feed conversion in groups 2 & 3. The total leukocytic and lymphocytic counts, besides the gamma and total globulin were proportionally increased in groups 2&3 at the end of the 6th week. Antibodies against Newcastle disease virus were enhanced at the end of the 6th week with phytase addition. Compared with the control group, feeding phytase elicited a significant dose-dependent increase in the phagocytic percentage and phagocytic index. The serum cholesterol and triglycerides were significantly decreased, while the serum phosphorus level was increased. Non-significant change was encountered in calcium and magnesium levels after phytase supplementation. These findings suggest that phytase enzyme causes dose-related improvement in chicken performance and stimulates their immunity without any harmful alteration in the studied biochemical parameters.

INTRODUCTION

Phytate is the form in which large portion of phosphorus (P) is present in plant feed ingredients. This makes it difficult for non-ruminants to gain their requirements out of being fed with these ingredients due to insufficient quantities or lack of intestinal phytase secretion *(1)*. Therefore, diets of mono-gastric animals are often supplemented with sources of inorganic Phosphate to meet the P requirements of the animal, which increases the cost of the diets and contributes to environmental pollution.

Dietary supplementation with sources of microbial phytase is well established as an effective and practical method of improving phytate digestibility in production animals *(2)*. Phytase hydrolyzes phytate to inositol and inorganic phosphate. This improves the availability of phytate-bound phosphorus from plant feedstuffs, thus lowers the need to add expensive commercial inorganic phosphate to poultry rations. Consequently, the cost of poultry feed and the lost P in excreta of intensive livestock operations are reduced *(3)*.

Phytase hydrolyzes the phosphate groups in phytates molecule, yielding lower molecular weight myoinositol phosphates, inositol, and inorganic P in the gastrointestinal (GI) tract *(4)*. The extra-phosphoric effects of dephosphorylation of phytates are not well elucidated and warrant further study because endogenous secretion, by the GI tract microflora, and the immune status of the host may be involved *(5)*. Therefore, it is interesting to understand the effect of phytase on the health and immune status of broilers

The objectives of the present study was to investigate the influence of phytase supplementation on the performance, immune functions and some biochemical parameters of broiler chickens fed corn-soybean based diets.

MATERIALS AND METHODS

1.Phytase enzyme (Natuphos)®

3-phytase from *Aspergillus Niger* produced by BASF Company, Germany (Natuphos 1000a/dry {a = FTU/gram}). Phytase activity is expressed as phytase units (FTU) per unit of feed which is a worldwide standard unit. One phytase unit is the activity of phytase that generates 1 micromole of inorganic phosphorus per minute from an excess of sodium phytate at pH 5.5 and 37 degrees Celsius.

2.Experimental chickens

Sixty one day old commercial Hubbard broiler chicken, were used. The chicken were reared under standard hygienic conditions and fed on corn-soybean based balanced commercial ration. All chickens were vaccinated against Newcastle disease at the age of 7 and 18 day and Gumboro disease at the age of 21 days. The chickens were equally divided into 3 groups. Gp. (1) was given a standard ration and kept as control. Gps. (2&3) were given a diet supplemented with 500 and 1000 unit of phytase /kg ration respectively for 6 weeks.

3.Growth performance

Chicks of all groups were weighed individually before the start of the experiment and at the end of the 3rd and 6th week from the start of experiment. Feed consumption per chick and feed conversion were calculated.

4.Blood sampling

Three blood samples were collected from each bird via heart puncture at the end of the 3rd and 6th week. Sample (1) was 1 ml of blood collected on EDTA for leukogram studies. Sample (2) was 2 ml of blood collected in a sterile plastic centrifuge tube containing heparin (50 IU/ml) to be used for cellular immune investigation. Sample (3) was 3 ml of blood taken without anticoagulant in a clean and dry centrifuge tube, left to clot at room temperature and then centrifuged at 3000 rpm for 5 minutes. The sera were collected for both biochemical and humoral immunological studies.

5.Leukogram studies

Leukocytic-counts were performed using an improved Neubaur Hemocytometer and Natt & Herrick solution. For differential leukocytic count, blood smears were prepared on clean slides, dried on air, fixed with absolute methanol and stained with Giemsa stain. The percentage and absolute values for each type of white cells were calculated *(6)*.

6.Humoral immune response

A) Proteinogram

Total protein was measured *(7)*. Electrophoretic analysis was carried out for determination of alpha, beta and gamma globulins according to the technique previously described *(8)*.

B) Hemagglutination inhibition antibody titer

The hemagglutination test (HA) was carried out *(9)* by using washed chicken erythrocytes with standard quantitative plate method to determine the end point of infected embryonic fluid. Micro-technique of hemagglutination inhibition test was adopted to Newcastle disease virus in serum samples *(10)*. Serial two fold dilutions of serum (1/2 to 1/1024) were prepared in 0.025ml volumes of saline. 4 HA unites of test virus in 0.025 ml volumes were added to each well and a reaction time of 10 minutes was allowed at 24°C before the addition of 0.5% RBCs suspension. Reading of end points was carried out after incubation for 30 minutes at 4°C and the titers were recorded. The highest serum dilution giving complete inhibition was the end point.

7.Cellular immune response

Phagocytic activity and phagocytic index

The heparinized blood samples were treated with 0.83% ammonium chloride to lyse the red blood cells. After washing by phosphate buffer solution, they resuspended in MEM to give final concentration of 108 viable polymorpho nuclears (PMN) cell/ml. A strain of coagulase-positive *Staph. aureus* suspension was adjusted to give final concentration of $5X10^8$ CFU/ml. 200 µl of this suspension was added to one ml of the PMN suspension to give a ratio of bacteria to PMN of 1:1. cell culture media using ultraviolet light-microscope *(11)*. The phagocytic-activity is considered as the percentage of phagocytic cells by microscope

field. The phagocytic-index is the mean number of *Staphylococcus aureus*, ingested by one phagocytic cell.

8.Clinicobiochemical studies

The serum total cholesterol was measured *(12)* and the serum triglycerides were estimated *(13)*. Serum calcium and magnesium were determined *(14)*. Serum inorganic phosphorus was determined *(15)*.

9. Statistical analysis

The obtained data were statistically analyzed by the Student's t-test *(16)*.

RESULTS AND DISCUSSION

The addition of microbial phytase to the diet of broiler chicken significantly improved all growth performance of gps. (2&3) in a linear manner in response to phytase dose (Table 1). These results agree with previous investigation *(17)* which reported that, the inclusion of 500 U phytase/kg diet improved weight gain at the age of 3 and 6 week by 6.7% and 6.1%, respectively. Similar results were also recorded *(18-21)*.

The growth-promoting effect of P, caused by phytase, can be partially attributed to the increased concentrations of myo-inositol, the final product of phytate desphosphorylation, and to the release minerals and trace elements from complexes with phytic acid. Similarly, it could be due to a possible increase of starch digestibility *(22)*, or an increased availability of protein *(23)*.

Phytase addition enhanced the bursa weight of 21-day-old Hubbard broilers *(24)*. The bursa is the source of B-cells, thus the development of the bursa may induce the proliferation of B-cells. The growth-promoting effect of phytase may be expressed via both nutrient release and a physiological regulation mechanism.

Regarding the leukogram finding, our results revealed a significant increase in total leukocytic and lymphocytic count in gps.(2&3) at the end of the 6th week of age (Table 2). These finding were associated with significant increase in the beta, gamma and total globulin, particularly in group 3 which was supplemented with the higher dose of phytase (Table 3). These results may be attributed to the immune-stimulating effect of phytase which markedly increased the lymphocyte numbers and the antibodies production *(5)*.

Serum hemagglutination inhibition antibody against NDV is a valid indicator for the humoral immunity of chickens *(25)*. In the current study, anti-NDV antibodies were improved by phytase addition, (Table 4) indicating that dietary factors may affect specific immune responses. There is some precedent for these effects in the literature *(5, 26)* as they proved that supplementation of diets with phytase enzyme preparations significantly increased the anti-NDV titers in chick.

The present study revealed a significant increase in the phagocytic percentage and phagocyte-index in both groups supplemented with phytase enzyme when compared with the control group (Table 4). Our results are in agreement with previous studies *(5, 27)* which reported that, the proliferation of lymphocytes and macrophage population were enhanced by phytase addition to the chicken diet which was found to be important in regulation of vital cellular functions, maturation and differentiation.

The biochemical results in our study revealed a significant decrease in the cholesterol and triglyceride levels in gps.(2&3) at the end of the 6th week of phytase supplementation (Table 5). Similar findings were recorded in broiler chickens after dietary microbial phytase addition at 500 or 1000 phytase units / kg ration *(28)*.

Seeds of different origins contain various complex interactions between phytate, minerals, starch, and protein *(29)*. When plant based diets are ingested, fermented within the crop, and then digested within the gastrointestinal tract, other complex interactions between dietary phytate, minerals, starch, and protein may potentially resulted in lower nutrient utilizations. The effects of phytase on the improvement of Ca and P utilization are well documented *(30)*.

Table 1. Growth performance (mean values ± S.E and % diff) in chickens of group 2 and 3 received a diet supplemented with 500 and 1000 unit of phytase enzyme/kg ration respectively for 6 weeks compared with control group

Time of sampling	Groups		Body weight gm	Feed consumption (gm/ bird)	Feed conversion
3rd week	Group 1 (control)	Mean ± SE	554 ± 9.8	956	1.73
	Group 2	Mean ± SE	582 ± 11.3	978	1.68
		% diff.	5.05%	2.3%	- 2.9%
	Group 3	Mean ± SE	596* ± 10.2	988	1.66
		% diff	7.6%	3.35%	- 4.05%
6th week	Group 1 (control)	Mean ± SE	1720 ± 25.2	3890	2.26
	Group 2	Mean ± SE	1846 *± 29.3	3980	2.16
		% diff	7.3%	2.2%	- 4.4%
	Group 3	Mean ± SE	1898** ± 32.4	4080	2.15
		% diff	10.35%	4.9%	- 4.87%

*Significant at P < 0.05 ** Significant at P < 0.01

Table 2. Leukogram (mean values ± S.E and % diff) in chickens of group 2 and 3 received a diet supplemented with 500 and 1000 unit of phytase enzyme/kg ration respectively for 6 weeks compared with control group

Time of sampling	Groups		T.L.C Cell x (10^3/µl)	Absolute differential count X (10^3/µl)				
				Lymphocytes	Heterophils	Monocytes	Eosinophils	Basophils
3rdweek	Group 1 (control)	Mean ± SE	12.9±0.3	4.52± o.12	6.26± 0.06	1.14±0.09	0.75±0.08	0.24±0.03
	Group 2	Mean ± SE	13.1±0.44	4.54± 0.09	6.33± 0.3	1.16±0.09	0.79± 0.11	0.26± 0.01
		% diff.	1.55%	0.58%	1.15%	1.58%	4.77%	10.17%
	Group 3	Mean ± SE	13.7± 0.22	4.82± 0.11	6.66± 0.18	1.18± 0.07	0.77± 0.03	0.26± 0.02
		% diff	6.2%	6.68%	6.46%	3.5%	1.59%	11.86%
6thweek	Group 1 (control)	Mean ± SE	13.15± 0.34	4.69± 0.19	6.49± 0.19	0.98± 0.11	0.76± 0.07	0.24± 0.03
	Group 2	Mean ± SE	14.28*±0.35	5.43*± 0.22	6.67±0.23	1.12± 0.05	0.78± 0.09	0.28± 0.04
		% diff	8.6%	15.8%	7.33%	14.11%	2.37%	14.8%
	Group 3	Mean ± SE	14.7*± 0.44	5.29*±0.14	6.78± 0.24	1.16± 0.06	0.88± 0.09	0.25± 0.03
		% diff	11.8%	12.8%	4.41%	18.6%	16.62%	3.31%

*Significant at P < 0.05

Table 3. Proteinogram (mean values ± S.E and % diff) in chickens of group 2 and 3 received a diet supplemented with 500 and 1000 unit of phytase enzyme/kg ration respectively for 6 weeks compared with control group

Time of sampling	Groups		Total protein (gm/dl)	Albumin (gm/dl)	α globulin (gm/dl)	β globulin (gm/dl)	γ globulin (gm/dl)	Total globulin (gm/dl)
3rd week	Group 1 (control)	Mean ± SE	3.66±0.21	1.94± 0.11	0.29± 0.02	0.49± 0.03	0.94± 0.06	1.72±0.11
	Group 2	Mean ± SE	3.9± 0.19	2.04± 0.12	0.27± 0.01	0.55± 0.02	1.04±0.05	1.86± 0.09
		% diff.	6.6%	5.15%	-6.9%	12.2%	10.6%	8.14%
	Group 3	Mean ± SE	4.2± 0.2	2.11± 0.11	0.3± 0.02	0.59*± 0.03	1.2*± 0.05	2.09*± 0.1
		% diff	14.7%	8.7%	3.4%	20.4%	27.6%	21.5%
6th week	Group 1 (control)	Mean ± SE	3.98± 0.22	2.23± 0.12	0.31± 0.02	0.5± 0.02	0.94± 0.05	1.75± 0.1
	Group 2	Mean ± SE	4.32± 0.16	2.22± 0.08	0.33± 0.02	0.61*± 0.03	1.16*±0.05	2.1*± 0.09
		% diff	8.5%	-0.4%	6.4%	22.0%	23.4%	20.0%
	Group 3	Mean ± SE	4.4± 0.17	2.21± 0.09	0.34± 0.01	0.63**±0.02	1.22**±0.05	2.18**±0.08
		% diff	10.55%	-0.8%	9.6%	26.0%	29.7%	24.5%

*Significant at P < 0.05 ** Significant at P < 0.01

Table 4. HI and cellular immunity (mean values ± S.E and % diff) in chickens of group 2 and 3 received a diet supplemented with 500 and 1000 unit of phytase enzyme/kg ration respectively for 6 weeks compared with control group

Time of sampling	Groups		HI against NDV	Phagocytic %	Phagocytic index
3rd week	Group1 (control)	Mean ± SE	4.0 ±0.25	74.6 ± 1.2	2.54± 0.06
	Group 2	Mean ± SE	4.2 ± 0.21	80.1 * ± 1.4	2.78*± 0.08
		% diff.	5 %	7.4 %	9.5%
	Group 3	Mean ± SE	4.34 ± 0.15	81.4 * ± 1.7	2.82*± 0.09
		% diff	8.5 %	9.1 %	11%
6th week	Group 1(control)	Mean ± SE	4.2 ± 0.21	76.8 ± 1.4	2.58± 0.07
	Group 2	Mean ± SE	4.8* ± 0.12	83.6 * ± 1.8	2.86*± 0.09
		% diff	14.3%	8.85 %	10.8%
	Group 3	Mean ± SE	4.92* ± 0.14	84.2 * ± 2.1	2.92*± 0.08
		% diff	17.1%	9.6 %	13.2%

*Significant at P < 0.05 ** Significant at P < 0.01
HI: haemagglutination inhibition

Table 5. Some biochemical parameters (mean values ± S.E and % diff) in chickens of group 2 and 3 received a diet supplemented with 500 and 1000 unit of phytase enzyme/kg ration respectively for 6 weeks compared with control group

Time of sampling	Groups		Cholesterol (mg/dl)	Triglycerides (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Magnesium (m mol/L)
3rdweek	Group 1 (control)	Mean ± SE	126.8 ± 3.9	81.8 ± 3.6	9.42± 0.43	4.23± 0.11	1.16± 0.11
	Group 2	Mean ± SE	120.3 ± 4.1	78.5 ± 2.8	9.36± 0.31	4.46 ± 0.13	1.22± 0.09
		% diff.	- 5.1%	- 4%	- 0.6%	5.4%	5.2%
	Group 3	Mean ± SE	118.2 ± 3.5	75.2 ± 3.7	9.32± 0.42	4.64*± 0.12	1.26± 0.08
		% diff	- 6.1 %	- 8%	- 1.06%	9.7%	8.6%
6thweek	Group 1 (control)	Mean ± SE	144.6 ± 3.4	91.8 ± 3.1	9.53± 0.3	4.27± 0.15	1.17± 0.09
	Group 2	Mean ± SE	132 *± 4.1	81.2*± 2.9	9.62± 0.39	4.68*± 0.08	1.25± 0.07
		% diff	-8.7 %	-11.6%	0.94%	9.6%	6.8%
	Group 3	Mean ± SE	129.2 *± 3.6	80.8*± 2.4	9.68 ± 0.44	4.74*± 0.09	1.29± 0.06
		% diff	-10.65%	-11.98%	1.57%	11%	10.25%

*Significant at P < 0.05

In the current study, the effects of phytase supplementation on serum mineral levels revealed non-significant change in calcium and magnesium levels with a significant increase in serum phosphorus level in gp. 3 at the end of the 3rd week and in both groups of phytase at the end of the 6th week. Our results were also consistent with those previously reported *(17, 31).*The absorption levels of Ca, P, Zn, Mn and Cu were increased by the addition of phytase enzyme. The results proved the positive effect of phytase, which is used for increasing the utilization rate of phytate P, Ca and other minerals in broilers *(32).* However, phytase supplementation was found to reduce plasma Ca level by 2.3%, in spite of obtaining an increase in Ca retention *(33,34).* These findings were explained by the fact that, chicks consuming the phytase-supplemented diets had restored homoeostatic balance between Ca and P as indicated by increasing levels of plasma P and slightly decreasing levels of plasma Ca.

As with Ca, plasma Mg concentration was reduced (5.3%) by phytase supplementation, even though Mg retention was increased *(35).* These contrasting results may be due to numbers of factors including phytase source, ingredients (type, source, phytate content) and dietary characteristics (processing, vitamin D3 and Ca:P ratio).

It could be concluded that, the phytase enzyme elicited a significant increase in body weight, improved feed conversion and stimulated immunity with a dose related manner without any harmful alteration in the studied biochemical parameters of the chickens.

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الملخص العربي

تأثير إضافة انزيم الفيتيز على الأداء الانتاجي، الوظائف المناعية و بعض القياسات البيوكيميائية في دجاج التسمين

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معهد بحوث صحة الحيوان – المعمل الفرعي بالزقازيق

اجري هذا البحث لدراسة تأثير اضافة انزيم الفيتيز إلى عليقة دجاج التسمين على الكفاءة الانتاجية و مناعة الدجاج و كذلك على بعض العناصر البيوكيميائية و قد استخدم لهذا الغرض عدد ٦٠ كتكوت تسمين عمر يوم تم تغذيتها على عليقة متوازنة تحتوي على الاحتياجات المقررة عالميا لمدة ستة اسابيع. تم تقسيم الكتاكيت بالتساوي الى ثلاثة مجموعات. المجموعة الأولى (مجموعة ضابطة) لم تتعرض عليقتها لأى اضافات. المجموعة الثانية و الثالثة اضيف لعليقتها انزيم الفيتيز (الناتوفوس) بنسبة ٥٠٠ ، ١٠٠٠ وحدة على التوالي لكل كجم عليقة من اليوم الاول و حتى نهاية الاسبوع السادس من عمر الكتاكيت. تم وزن الدجاج و حساب معدل استهلاك الغذاء و معدل التحويل الغذائي و كذلك تجميع عينات الدم من كل مجموعة عند نهاية الاسبوع الثالث و السادس من التجربة.

اظهرت النتائج ان اضافة انزيم الفيتيز الى عليقة الدجاج احدثت زيادة معنوية في وزن الدجاج و تحسن في مستوى الكفاءة التحويلية للغذاء فى المجموعتين (٢&٣) عند نهاية الاسبوع السادس. و عند فحص الدم كانت هناك زيادة معنوية في العدد الكلي لكرات الدم البيضاء و الكريات اللمفية. و باجراء اختبار الفصل الكهربي للبروتين وجد زيادة معنوية في الجاما جلوبيولين و الجلوبيولين الكلي و كانت هذه الزيادة تتناسب طرديا مع زيادة الجرعة المستخدمة. كما اوضحت نتائج الاختبارات المناعية وجود زيادة معنوية بالنسبة لاختبار التلازن و ايضا نسبة التهام البكتريا وكانت هذه الزيادة اكثر و ضوحا في المجموعة الثالثة عند استخدام التركيز الاعلى من انزيم الفيتيز.

اظهر فحص السيرم نقصا معنويا في نسبة الكوليستيرول و الجليسريدات الثلاثية مصحوبا بزيادة معنوية فى معدلات الفوسفور في السيرم بينما لم يحدث تغيرات معنوية في مستويات الكالسيوم و الماغنسيوم فى المجموعتين (٢&٣) مقارنة بالمجموعة الضابطة.

من هذه النتائج نستخلص ان اضافة انزيم الفيتيز الى عليقة دجاج التسمين كان له تأثيرا ايجابيا على وزن الدجاج و معامل التحويل الغذائي و كذلك الاستجابة المناعية والعناصر البيوكيميائية التي تم دراستها بدون تسجيل اثار جانبية.