

## Effect of Different Levels of Phytase in Laying Hen Diets on Some Biochemical Blood Parameters

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### Abstract

The present study was carried out to evaluate the effect of adding two levels of enzymes (0 and 0.8 kg / ton diet) such as phytase (0.3 kg/ ton) and glucanase (0.5kg/ ton) and substitute barley by corn in laying diet with the levels of 0, 25 and 50% as a replace of corn on some physiological responses. A total number (288) of males and females Anshass layer chicks, five weeks old were used in this study (72 of males and 216 of females). Chicks were randomly distributed into 6 groups. Each group contains 4 replicates of 12 males and 36 females, each. The experiment was lasted at 20 weeks of age. The data revealed that, Adding enzyme supplementation to layer diets contain different levels of barley had no significant effect ( $P \geq 0.05$ ) on total protein, total albumin, globulin, glucose, GOT, GPT and total lipids. While, it could be noticed that birds fed dietary enzyme recorded a slightly decreased ( $P \geq 0.05$ ) in total protein, total albumin, and glucose compared with others fed un-supplemented diet. However, layers fed dietary enzyme recorded a slightly enhancement in total globulin compared with other fed control diet. Moreover, Layers fed 25% dietary barley with enzyme addition recorded a slightly improvement ( $P \geq 0.05$ ) in GOT values, though, this enhancement was noticed in GPT values for birds fed control diet with enzyme.

**Keywords:** Enzymes, Layer Chicks, Blood Parameters.

### Introduction

In Egypt, the consumption of poultry meat and egg has consistently increased over the years. This trend is expected to continue because there is a change in the eating patterns of the populace leading to a greater demand for poultry products. However, the poultry industry in Egypt is confronted with a number of challenges, especially the pressures to produce high quality products to satisfy customer needs in a cost effective manner. In poultry productions, feed cost has always been one of the major issues; accounting for up to 70% of total production Cost [1]. The high cost of feeding is attributed to the high cost and scarcity of conventional feed ingredients like maize, groundnut cake, soybean meal and fish meal. A possible approach to reducing the relatively high feed cost in commercial laying hen production is the utilization untraditional ingredients in laying hen diets such as barley instead of corn. This is necessary since the production levels of conventional feed ingredients are not increasing proportionately to meet the increasing demand. The major components of barley are starch, dietary fiber, and crude in digestible energy content.

Barley is utilized to best advantage in poultry diets when consideration is given to the class of bird being fed and the desired level of performance. The  $\beta$ -glucans inherent to most barley can create problems for young birds and birds raised on litter. The amount of barley in broiler diets can gradually be increased as the birds

become adapted to it. When poultry are fed barley based diets, reduced variability in performance and improved growth can be obtained through the use of enzyme supplementation. During peak production period's barley should not be used as the sole grain components.

Exogenous enzyme has long been used in poultry industry to alleviate the anti-nutritional factors and improve the utilization of dietary energy and protein, thus leading to enhanced poultry performance [2]. Due to the structural complexity in feedstuffs, it has been pointed out that the multiple enzyme preparations with the substrate-specific activities be regarded as the next generation of technology. Phytase enzyme has been added to poultry diets as exogenous phytase. A high or low level of available P in a laying hen's diet may adversely affect the bird's performance and reduce the eggshell quality. The use of phytase in layer diets improves phytate P utilization and reduces the requirement for inorganic P. (Gordon and reported that hens consuming the low nonphytate P (NPP) diet with supplementary phytase performed as well as the hens fed diets containing higher levels of NPP without supplementary phytase. However, the effects of phytase in layer diets are complicated by the intimate link between Ca and P metabolism. Compared to broiler chicks, phytase inclusion in diets for laying hens has been the subject of less research.

The aim of this study was to evaluate the effect of adding two levels of enzymes mixture (control and 300g phytase plus 500gcocktail enzyme) and substitute barley by corn in laying diet with the levels of 0, 25 and 50% on some physiological responses.

### Materials and methods

The present study was carried out at the farm of Animal and Poultry Production, Faculty of Agriculture, Minia University. It was designed to investigate the effect of enzyme addition at levels of (0 and 300 gm phytase and 500 gm cocktail enzyme/ton) into different dietary treatments based on replacing barley instead of ground corn by the levels of (0, 25, 50%) in a factorial arrangement on growth performance, egg production and some blood constituents.

### Experimental chicks and housing

A total number (288) Anshass layers chicks (72 of males and 216 of females), five weeks old were used in this study. Chicks were obtained from Azab Company in Fayoum, Egypt. Chicks were randomly distributed into 6 groups (12 male and 36 female). Each group contains 4 replicates of 3 males and 9 females, each. All chicks were housed in two-tiers floor batteries located in an open house. The dimensions of the cage in each battery were (100 ×60 × 40cm) for length, width and height, respectively. The batteries were previously cleaned and disinfected. Average initial body weight of all treatments was almost similar. The experiment was lasted at 20 weeks of age [4-7].

### Experimental diets

Three diets within each of the grower and finisher diets were formulated to have recommended levels of both crude protein and metabolizable energy. The first grower and finisher diets were formulated to meet the nutrients requirements of laying hens according to NRC (1994) and was served as a control treatment. The second and third grower and finisher diets were formulated to have replaced barley instead of ground corn by the levels of (25 and 50%) with or without adding enzyme. All diets were arranged in a factorial arrangement to be two levels of enzymes (0 and 300 gm phytase plus 500 gm cocktail enzyme/ton) were added into different dietary treatments which based on replacing barley instead of ground corn by the levels of (0, 25, 50%). The cocktail enzyme enzymes used in this experiment contain : Endo1,3(4)-beta- glucanase (beta- glucanase 1175 units /g), Endo1,4-beta- glucanase (cellulose 2000 units/ g),alpha amylase 200 units/g, bacillomycin (protease)225 units/g, and endo – 1,4- beta xylanase(xylanase 10000 units/ g. The ingredients and proximate analyses of the dietary treatments for chickens fed from 5 to 8 and from 9 to 25weeks of age are presented in Tables (1) and (2).

### Chicks management

All groups were randomly allocated in batteries and kept under similar conditions of management. Artificial lighting was provided 12 hours daily until first egg of layers, then the artificial light was used in the evening to complete 17 hours per day.

**Table 1: The ingredients and proximate analyses of the dietary treatments for chickens fed from 5 to 8 weeks of age.**

Ingredients	Treatments					
	Enzyme level					
	Without enzyme (0%)			300 phytase+500 cocktail gm/ ton )(		
	0% Bar.	25% Bar.	50%Bar.	0% Bar.	25% Bar.	50%Bar.
Yellow corn	62	46.50	31	62	46.50	31
Barley	0	15.50	31	0	15.50	31
Soybean meal	20.8	20.8	20.8	20.8	20.8	20.8
layer conc., 49% CP	10	10	10	10	10	10
Limestone	6	6	6	6	6	6
Nacl( salt)	0.7	0.7	0.7	0.7	0.7	0.7
Premix**	0.5	0.5	0.5	0.5	0.5	0.5
Proximate analysis						
Metabolizable energy K cal/kg	2759.74	2649.69	2539.64	2759.74	2649.69	2539.64
Crude protein,	19.01	19.48	19.94	19.01	19.48	19.94
Calcium, %	2.68	2.68	2.68	2.68	2.68	2.68
Available phosphorus,	0.55	0.56	0.57	0.55	0.56	0.57
Methionine and cysteine	0.75	0.76	0.77	0.75	0.76	0.77
Lysine, %	0.94	0.96	0.98	0.94	0.96	0.98

\*Each 1 kg Premix contained: Vit A 3350000 IU Vit D3 760000 IU Vit E 6700 IU Vit K3 335 mg Vit B1 334 mg Vit B2 1670 mg Vit B6 500 mg Vit B12 3.4 mg Niacin 10 000 mg Ca.D.Pantothenate 3334 mg Biotin 16.7 mg Folic acid 334 mg, Trace minerals: Iron 13 350 mg Copper 3 335 mg Zinc 16 700 mg Manganese 25000 mg Iodine 500 mg Cobalt 84 mg Selenium 100 mg, Additives: Ethoxyquine 600 mg, Carrier ( ca co<sub>3</sub>) up to 1 kg.

**Table 2: The ingredients and proximate analyses of the dietary treatments for chickens fed from 9 to 25 weeks of age.**

Ingredients	Treatments					
	Enzyme level					
	Without enzyme (0%)			300 phytase+500 cocktail gm/ ton )(		
	0% Bar.	25% Bar.	50%Bar.	0% Bar.	25% Bar.	50%Bar.
Yellow corn	70	52.50	35	70	52.50	35
Barley	0	17.50	35	0	17.50	35
Soybean meal	15.8	15.8	15.8	15.8	15.8	15.8
layer conc., 49% CP	7	7	7	7	7	7
Limestone	6	6	6	6	6	6
Nacl( salt)	0.7	0.7	0.7	0.7	0.7	0.7
Premix**	0.5	0.5	0.5	0.5	0.5	0.5
Proximate analysis						
Metabolizable energy K cal/kg	2850.57	2726.32	2602.07	2850.57	2726.32	2602.07
Crude protein,	15.98	16.50	17.03	15.98	16.50	17.03
Calcium, %	2.57	2.57	2.57	2.57	2.57	2.57
Available phosphorus,	0.47	0.48	0.49	0.47	0.48	0.49
Methionine and cysteine	0.63	0.65	0.66	0.63	0.65	0.66
Lysine, %	0.76	0.78	0.81	0.76	0.78	0.81

\*Each 1 kg Premix contained: Vit A 3350000 IU Vit D 3760000 IU Vit E 6700 IU Vit K 3335 mg Vit B 1334 mg Vit B 21670 mg Vit B 6500 mg Vit B 123.4 mg Niacin 10000 mg Ca.D.Pantothenate 3334 mg Biotin 16.7 mg Folic acid 334 mg, Trace minerals: Iron 13350 mg Copper 3335 mg Zinc 16700 mg Manganese 25000 mg Iodine 500 mg Cobalt 84 mg Selenium 100 mg, Additives: Ethoxyquin 600 mg, Carrier (ca co<sub>3</sub>) up to 1 kg.

### Vaccination program

The chicks were vaccinated against the common poultry diseases as follow:

**Table 3: Types of vaccinations of chicks**

Age (day)	Vaccine	Method of vaccination
7	Hitchner - B1	Drinking water
9	Me fuvah5+ND	Injection
14	Gambaro 1	Drinking water
18	Gambaro 1	Drinking water
20	Colona IB	Injection
26	Lasota	Drinking water

### Some blood parameters:

At the end of the productive period of the experiment (25th week of age) , blood samples were collected from each experimental group. The blood was taken from the chicken wing vein in tubes to obtain plasma. The plasma was separated by centrifugation at 3000 rpm for 20 minutes and stored at -20 °C until analysis.

### Serum total protein (gm/ dl):

Serum total protein was determined according to the method of (Gornal et al., 1949) using reagent kits purchased from biodiagnostic chemical company (Egypt).

Serum albumin (gm/dl):

Serum albumin concentration was determined according to the

method of using reagent kits purchase from diamond diagnostics chemical company (Egypt).

Serum globulin (gm/dl):

Serum globulin concentration was calculated according to using the following equation:-

Globulin (g/dl) = Total protein (g/dl) – Albumin (g/dl)

Serum albumin/globulin ratio (A/G ratio):

Serum albumin/globulin ratio was calculated as follow:-

$$\text{Albumin/ globulin ratio} = \frac{\text{Albumin value (g/dl)}}{\text{Globulin value (g/dl)}}$$

### Serum glucose (mg/dl):

Serum glucose concentration was determined according to the method of (using reagent kits purchase from biodiagnostic chemical company (Egypt).

### Serum total lipids (gm/dl):

Serum total lipid was determined according to using reagent kits purchased from biodiagnostic chemical company (Egypt).

### Determination of liver enzymes:

GPT (ALT) glutamic pyruvic transaminase

Glutamic-Pyruvic Transaminase activity in serum was determined according to using reagent kits purchased from biodiagnostic chemical company (Egypt).

### GOT (AST) glutamic oxaloacetic transaminase

Glutamic- Oxaloacetic Transaminase activity in serum was determined according to using reagent kits purchase from biodiagnostic chemical company (Egypt) [8, 14].

### Statistical analysis

Data were statistically analyzed by the analysis of variance using the General Linear Model (GLM) procedure of Statistical Analysis System) (SAS, 1998). Significant differences among treatments were separated by Duncan's multiple range tests Duncan (1955).

The following statistical model was used.

$$Y_{ijk} = M + D_i + E_j + DE_{ij} + e_{ijk}$$

Where:

$Y_{ijk}$  = an observation measured.

$M$  = the overall mean.

$D_i$  = effect of barley (1, 2, 3)

$E_j$  = effect of enzymes (1, 2).

$DE_{ij}$  = effect of interaction ( $D_i \times E_j$ ).

$e_{ijk}$  = experimental error.

## Results and Discussion

Total protein, Total albumin, globulin and glucose.

### Effects of enzymes:

Averages values of total protein, total albumin, globulin and glucose  $\pm$  SE at the end of the experiment (25 weeks of age) as affected by enzyme supplementation into laying diet based on substitute barley by corn at different levels are presented in (Table 4). The data revealed that, adding enzyme to growing layer diets had no significant effect ( $P \geq 0.05$ ) on total protein, total albumin, globulin and glucose. While, it could be noticed that birds fed dietary enzyme recorded a slightly decreased ( $P \geq 0.05$ ) in total protein, total albumin, and glucose compared with others fed un-supplemented diet. However, layers fed dietary enzyme recorded a slightly enhancement in total globulin compared with other fed control diet.

The enhancement in total protein, albumin and glucose as a result of using enzymes may be due to that, the effect of phytase on amino acid digestibility tends to be greatest on those amino acids that are prevalent in intestinal maintenance and turnover, specifically cystine, threonine, proline and glycine when measured. This suggests that a greater proportion of the benefit of phytase is due to reduced endogenous losses rather than increased dietary amino acid digestibility. Thus, destruction of phytate reduces the anti-nutritive effect in a directly proportional manner and as a result, energy and amino acids that would have been used in a maintenance activity can be directed towards productive energy (growth) instead. Illustrated that serum total protein and albumin concentrations were increased by phytase addition to broiler chick diets. Reported that enzyme supplementation (ZADO) which contain xylanase, cellulase, protease and  $\alpha$ -amylase to broiler chick diets increased plasma total protein and globulin at 42 days of age.

**Table 4: Effect of dietary phytase, barley supplementation and their interaction on blood (ml/dl) of laying chick**

Treatments	Items(ml/dl) Table (4). Effect of dietary phytase, barley supplementation and their interaction on blood (ml/dl) of laying chick						
	Glucose(ml/dl)	Total Lipids(ml/dl)	GPT(IU/L)	GOT(IU/L)	Globulin(ml/dl)	Albumin (ml/dl)	Total Protein(ml/dl)
A) Effect of enzyme							
Sig	NS	NS	NS	NS	NS	NS	NS
Without enzyme	4.945	2.145	2.145	149.300	6.791	6.791	263.425
With enzyme	5.068	2.540	2.540	168.100	7.816	7.816	300.591
$\pm$ SE	0.297	0.166	0.166	6.643	0.448	0.448	14.437
B) Effect of barley							
Sig	NS	NS	NS	NS	NS	NS	NS
Control without barley	5.485	2.496	2.988	155.875	7.525	1.016	277.312
.25% Barely0	4.8562	2.295	2.732	162.200	7.512	1.0262	275.475
0.5% Barely	4.680	2.237	2.494	158.025	6.875	1.030	293.237

± SE	0.364	0.203	0.366	8.137	0.549	0.052	17.682
(A*B)Interaction							
Sig	NS	NS	NS	NS	NS	NS	NS
Without enzyme control	5.752	2.6425	3.110	141.500	6.850	1.1050	269.25
Without enzyme 0.25%	4.595	2.0125	2.65250	149.975	7.3	0.9875	258.65
Without en-zyme0.5%	4.49	1.780	2.7875	156.425	6.225	1.0475	262.37
With enzyme control	5.2175	2.350	2.8675	170.250	8.2	0.9275	285.375
With enzyme 0.25%	5.1175	2.5775	2.8125	174.425	7.725	1.065	292.3
With en-zyme0.5%	4.87	2.6950	2.2000	159.625	7.525	1.0125	324.1
± SE	0.516	0.287	0.5177	11.507	0.7768	0.074	25.007

a-b Values within columns with no common superscripts are significantly different ( $P < 0.05$ ).

(A)= Enzyme Effect, (B) = Effect of BARLY addition, (A\*B) = Interaction Effect.

### Effects of dietary barley

Averages values of total protein, total albumin, globulin and glucose  $\pm$ SE at the end of the experiment (25 weeks of age) as affected by dietary barley at the levels (0, 25 and 50% barley as a substitute of corn) are presented in (Table 4). The data revealed that, using barley as a substitute of corn in layer diets at levels either 0 or 25% and 50% had no significantly effect ( $P \leq 0.05$ ) on total protein, total albumin, globulin and glucose. However, layers fed control diet recorded the greatest ( $P \leq 0.05$ ) values of total protein; total albumin and globulin followed by layers fed 25% dietary barley compared with 50% barley. Also, it could be noticed the layers fed 50% dietary barley recorded a slightly ( $P \leq 0.05$ ) enhancement in glucose value followed by control diet compared with layers fed 25% dietary barley.

The previous results are agreeing with the finding of , found that corn substitution by wheat grain at 25% or 50% non-significantly reduced total protein concentration and increased ( $P \geq 0.05$ ) blood serum albumin and globulin concentrations when compared with broiler chicken fed on corn – soybean based diet. Moreover, enzyme supplementation in wheat grain containing ration had no significant on blood serum total protein, albumin and globulin concentrations when compared with broiler chicken group fed on the same diet without enzyme supplementation. Regarding blood serum glucose concentration, it was noticed that corn substitution by wheat grain and enzyme supplementation in broiler diet based on corn – soybean or containing different levels of wheat grain had no significant effect on blood serum glucose [14, 19].

### Effect of the interaction between enzyme addition and dietary barley

Averages values of total protein, total albumin, globulin and glucose at the end of the experiment (25 weeks of age) as affected by the interaction between enzyme addition and dietary barley are presented in (Table 4).The data revealed that, adding enzyme supplementation at levels of (0 and 300 gm phytase and 500 gm cocktail enzyme /ton ) to layer diets contain different levels of barley

(0, 25 and 50% barley as a substitute of corn) had no significant effect ( $P \geq 0.05$ ) on total protein, total albumin, globulin and glucose. In general, chicks fed control diet without enzyme addition recorded a slightly improvement ( $P \geq 0.05$ ) in total protein, total albumin, and globulin compared with other levels of dietary barley incorporated with enzyme addition .Layers fed all levels of barley diets with enzyme supplementation recorded a slightly enhancement ( $P \geq 0.05$ ) in glucose compared with other dietary treatments either without enzyme addition .

The improvement of glucose as a result of adding enzyme may be due to the improvements in nutrients digestibility by enzyme supplementation which can be a reason for increased blood glucose concentration in broiler chicks [20]. The previous results are agreeing with the finding showed that, turkey plasma total protein, albumin and globulin concentration were not differing significantly as birds fed barley based-diets supplemented with or without enzymes (optizyme-p5) 1kg/ ton.

Hajati et al., (2009) and Hajati (2010) found that, adding enzymes (Endo feed w + xylanase + $\beta$ -glucanase) to broiler chick diets increased blood glucose at 44 days of age. However, Jalali and Babaei (2012) illustrated that serum total protein and albumin concentrations were increased by phytase addition to broiler chick diets. Safaa (2013) reported that enzyme supplementation (ZADO) which contains xylanase, cellulase, protease and  $\alpha$ -amylase to broiler chick diets increased plasma total protein and globulin at 42 days of age.

### Liver function:

Liver function (glutamate-oxaloacetate transaminase (GOT), (GPT) glutamate-pyruvate transaminase) and total lipids

### Effects of enzymes

Averages values of GOT, GPT and total lipids  $\pm$ SE at the end of the experiment (25 weeks of age) as affected by enzyme supplementation into laying diet based on substitute barley by corn at different

levels are presented in (Table 4). The data revealed that, adding enzyme to growing layer diets had no significant effect ( $P \geq 0.05$ ) on GOT, GPT and total lipids. While, it could be noticed that birds fed dietary enzyme recorded a slightly increased ( $P \geq 0.05$ ) in GOT and GPT compared with others fed un-supplemented diet. However, layers fed dietary enzyme recorded a slightly enhancement ( $P \geq 0.05$ ) in total lipids compared with other fed control diet (un-supplemented with enzyme).

The present data are supported by those obtained by El-Katcha, et al., (2014), found that enzyme supplementation (both kemzyme plus or combozyme products) had no significant effect on blood serum GOT and GPT concentrations when compared with broiler chicken groups fed on the same diet without enzyme supplementation. Also, obtained by Lee et al. (2010) who indicated that there were no significant differences in the activities of serum GOT and GPT among control and enzyme treated groups of broiler chickens. Measurement of GOT and GPT activities are indicative of liver damage in broiler chicks and is therefore a valuable tool for determination of a safe inclusion rate for feed additives.

### Effects of dietary barley

Averages values of GOT, GPT and total lipids  $\pm$ SE at the end of the experiment (25 weeks of age) as affected by dietary barley at the levels (0, 25 and 50% barley as a substitute of corn) are presented in (Table 4). The data revealed that, using barley as a substitute of corn in layer diets at levels either 0 or 25% and 50% had no significant effect ( $P \leq 0.05$ ) on GOT, GPT and total lipids.

The previous results are agreed with the finding of found that revealed that, wheat grain inclusion at 25 or 50% from corn content of broiler chicken ration non significantly ( $P \geq 0.05$ ) increased blood serum GPT concentration by about 8.0% and 17.5% respectively. These data indicated that wheat inclusion in broiler chicken ration had adverse effect on hepatic cell and broiler wheat inclusion as substitute of corn grain at 25 or 50% non-significantly reduced blood serum triglyceride, total cholesterol and high density lipoprotein (HDL) concentrations by about (13.3% and 16.8%), (3.4% and 6.9%) and (6.8% and 36.8%) respectively when compared with chicken group fed on corn soy bean based diet without enzyme supplementation. However, 25% and 50% of corn replacement by wheat grain without enzyme supplementation significantly increased blood serum GOT concentration of broiler chicken by about 94.8% and 272.9% respectively when compared with broiler chick group fed on corn-soybean based diet without enzyme supplementation.

### Effect of the interaction between enzyme addition and dietary barley

Averages values of GOT, GPT and total lipids at the end of the experiment (25 weeks of age) as affected by the interaction between enzyme addition and dietary barley are presented in (Table 4). The data revealed that, adding enzyme supplementation at levels of (0 and 300 gm phytase and 500 gm cocktail enzyme /ton ) to layer

diets contain different levels of barley (0, 25 and 50% barley as a substitute of corn) had no significant effect ( $P \geq 0.05$ ) on GOT, GPT and total lipids. Layers fed 25% dietary barley with enzyme addition recorded a slightly improvement ( $P \geq 0.05$ ) in GOT values, however, this enhancement was noticed in GPT values for birds fed control diet with enzyme. The total lipids values was improved when chicks fed control diet without enzyme compared with other dietary treatments either without enzyme addition.

Abd El hameed, (2014) reported that birds fed dietary 5%, 15% DDGS without enzyme and 10 % with enzyme recorded the greatest significant ( $P \leq 0.01$ ) values of GOT and GPT compared to other groups. While, birds fed control diet was recorded the lowest value of GPT compared to other treatments at the end of experiment. It could be concluded that, adding DDGS up to 15% in broiler diet. It could be concluded that, adding DDGS up to 15% in broiler diet had no adverse effect on some blood biochemical parameters. Gao et al. (2008) reported that, xylanase supplementation to wheat-based diets increased the concentration of blood Insulin-Like Growth Factor (IGF-I) and insulin of 21-day-old broilers, which indicated that enhanced digestion and absorption of nutrients, caused by the enzyme supplementation, could have an effect on hormone concentrations. Lee et al. (2010) they stated that enzyme supplementation had no significant effect on blood serum cholesterol concentration of broiler chickens. Glucans may reduce the absorption of fat and cholesterol and are known to have cholesterol lowering properties. These effects are all associated with the viscosity forming properties of soluble dietary fibers [21-29].

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