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EFFECT OF SUPPLEMENTING DIFFERENT LEVELS AND SOURCES OF PHYTASE ENZYME TO THE LAYING HENS DIETS ON PRODUCTIVE PERFORMANCE

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Abstract

This study was conducted in the Poultry Research Station – State Board of Agricultural Research - Ministry of Agricultural in Abu Ghraib, to study the effect of supplementing different levels and sources of Phytase enzyme to laying hens diets on productive performance and quality of eggs produced .

Nine hundred sixty egg-brown hens (Lohmann Brown-Classic) 24 weeks-old distributed randomly to six treatments with various sources of phytase, each source contain three levels which include 16 treatments with 2 replicates (30 hen per replicate). Chickens fed on diets equal protein and metabolic energy according to the age periods in Lohmann Index as in follow:

Treatment 1 (T1): Control group (without any supplement and without reduction of calcium and phosphorus).
Treatment 2 (T2): Supplement phytase from fungal source (Aspergillus oryza).
Treatment 3 (T3): Supplement phytase from bacterial source (E. coli).
Treatment 4 (T4): Supplement phytase from phytase mixture
For every three source of phytase added 3 levels as follows:
A: a diet reduced phosphorus (0.09) and calcium (0.07) which included Phytase adding by 250 FTY / kg feed
B: a diet reduced phosphorus (0.12) and calcium (0.10) which included Phytase adding by 350 FTY / kg feed
C: a diet reduced phosphorus (0.15) and calcium (0.13) which included Phytase adding by 450 FTY / kg feed
Treatment 5 (T5): Supplement Phytase from yeast (Saccharomyces cerevisia).
Treatment 6 (T6): Supplement Phytase from Alfalfa plant.
For every two source of Phytase (T5 and T6) added 3 levels 2.5, 3.5 and 4.5 FTY for each source. All three levels diet reduced phosphorus and calcium as in T2, T3 and T4.
The results of experiment showed:
Significant differences (P<0.05) between treatments in accumulative egg production (HD%), egg weight, egg mass and feed conversion ratio, while the adding of T2 treatment at level (A,C) and T4 treatment at level(C) were highly significant than other treatments.
All treatments with adding Phytase enzyme showed significant superiority (P<0.05) for qualitative characteristics of eggs produced from 24-55 weeks as follows.
From this study we can concluded that supplementing fungal phytase at levels 250 and 450 FTY/kg feed in layer diets with decrease phosphore 0.09 and 0.15 % and calcium 0.07 and 0.13% respectively gave the best results also when supplementing mixture enzyme at level 450 FTY/kg feed.

Key words: *phytase, laying hens, productive performances, eggs quality.*

INTRODUCTION

The poultry industry at the present time faced many environmental problem, like poultry waste that accumulation in very large quantities (Toth et. al., 2006). China produced nearly 2.21 billion tons of poultry waste in 2003 (Huang et. al., 2006) and its content 40% organic material.

Environmental pollution with phosphorus increased attention by the associations of environmental protection, especially if environmental pollution sources of this element from poultry manure over and above rainwater and sewage that have a role in this pollution (Howarth et al., 2002; Sim and Sharpley, 2005).

All of this is linked to the operations of poultry feeding, where the form of cereal crops and their by-products and oil seeds main ingredients in diets because of nutritional value, these components contain phosphorus element which has importance in metabolism as a source of energy through the entry in the synthesis of ATP and creatine phosphate also being primarily created in nucleic acids DNA and RNA and responsible for phosphorylation of some nutrients during metabolism as well as its entry in the synthesis of many compounds such as co-carboxylase, phosphokinase and Vit.B1 (Thiamine pyrophosphate) (Hatten et al., 2001).

Two-thirds of phosphorus in poultry diets be a complex compound unavailable form known as phytic acid (organic phosphorus) (Lott et al., 2000; Gibson et al., 2010).

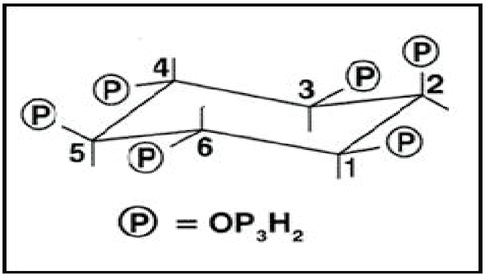

Fig.1 The chemical composition of phytic acid (Kumar et al., 2010)

Due to the inability of poultry to use phytate phosphorus to the lack of phytase enzyme that responsible for smashing phosphorus bond from inositol ring and make it available for mono gastric animals (Singh et al., 2007).

This has led the developed countries to adding phytase enzyme to poultry diets in order to reduce phosphorus in feces (Francesch et al, 2005; Ciftci et al., 2005). Studies have shown the importance of phytase enzyme to reduce the ratio of phosphorus in poultry feces to more than 30% (Pulmstead, 2007).

Musapuor et al. (2005) explained that the addition of phytase enzyme to the laying diets have a positive impact in improving the performance and the production of eggs ,also added to diets poor with phosphorus improved the level of calcium and phosphorus and egg weight and production (Scott et al., 1999).

Snow et al. (2003) pointed that laying hens fed on phytase enzyme by 300FTY/kg for 21 days did not have any significant effect on egg production. While the addition of Silversides and Hurby (2009) from phytase enzyme at levels 300 and 600FTY/kg to Lohmann hens with presence of phosphorus at perfect level doesn't appear any effect in phosphorus, calcium, energy and protein so from this results the researchers concluded that the addition of phytase enzyme to poor diets can ensuring necessity body from phosphorus and reduce phosphorus excreted with feces and thus reduce environmental pollution. Haitham (2010) found that using phytase enzyme Natophus and Phyzyme at level 300 and 450 respectively (two different microbial sources of phytase enzyme) to corn-soybean diets have a positive impact on the productive performance, feed efficiency and egg weight. Also Mohebbifar and Torki (2011) revealed that using 0.3 gm from phytase enzyme /kg at levels 0.33 and 0.29 gm from phytate phosphorus /gm led to significant increase in egg production due to increased feed consumption and feed conversion ratio compared with control treatment.

The aim of this study added different sources of phytase enzyme and different levels to laying hen diets and their effects on productive performance.

MATERIALS AND METHODS

Using 960 Lohmann Brown-Classic laying hens at 24 week old distributed randomly on 16 treatment (2 replicate / treatment)30 hen per replicate .Treatments were as follows:

T1: Treatment 1 as Control (without adding enzyme and non-reduced phosphorus and calcium).

T2: Treatment 2 adding phytase enzyme from fungal source.

T3: Treatment 3 adding phytase enzyme from bacterial source.

T4: Treatment 4 adding phytase enzyme from enzyme mixture.

Treatments 2,3,4 adding by three levels as in follow:

Level A: Diet reduced phosphorus (0.09%) and calcium (0.07%) with adding 250 FTY phytase enzyme /kg feed.

Level B: Diet reduced phosphorus (0.12%) and calcium (0.10%) with adding 350 FTY phytase enzyme /kg feed.

Level C: Diet reduced phosphorus (0.15%) and calcium (0.13%) with adding 450 FTY phytase enzyme/kg feed.

T5: Treatment 5 adding phytase enzyme from yeast source.

T6: Treatment 6 adding phytase enzyme from plant source.

Treatments 5 and 6 adding by three levels as in follow:

Level A: Diet reduced phosphorus (0.09%) and calcium (0.07%) with adding 2.5 FTY phytase enzyme /kg feed.

Level B: Diet reduced phosphorus (0.12%) and calcium (0.10%) with adding 3.5 FTY phytase enzyme /kg feed.

Level C: Diet reduced phosphorus (0.15%) and calcium (0.13%) with adding 4.5 FTY phytase enzyme/kg feed.

Tables 1, 2, 3 indicate the diets used in the experiment for the periods from 18-28, 29-45, 46-63 weeks of age.

Table 1. Components and chemical composition (%) of the diet used in laying hens from age 18 to 28 week

Treatment / **Components**	**T1 control**	**T2,T3, T4(1)**			**(S.C)T5**			**T6(ALFALFA)**		
		A FTY 250	**B FTV 350**	**C FTY 450**	**A FTY 2.5**	**B FTY 3.5**	**C FTY 4.5**	**A FTY 2.5**	**B FTY 3.5**	**C FTY 4.5**
Corn	53	53	50.89	53.02	53	52.081	52	52.047	50.49	50
wheat	7.34	7.71	10	8.21	8.466	9.37	9.11	7.59	9.1	9
SBM (44% cp)	24	24.02	24	24	23.208	23.32	23.659	23.437	22.862	22.91
Protein Centre (2)	5	5	5	5	5	5	5	5	5	5
fat	2	2	2	1.8	1.9	1.9	2	2.143	2.32	2.407
Di-calcium phosphate (18.5 P)	1.75	1.25	1.07	0.91	1.25	107	0.91	1.25	1.07	0.91
Calcium carbonate (limestone)	6.82	6.93	6.95	6.97	6.93	6.95	6.95	6.88	6.88	6.87
NaCI	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Yeast S.C	0	0	0	0	0.156	0.219	0.281	0	0	0
Dried leaves alfalfa	0	0	0	0	0	0	0	1.563	2.188	2.813
Total	100	100	100	100	100	100	100	100	100	100
Calculated Chemical analysis(3)										
Energy (kcal I kg)	2804	2825	2811	2812	2813	2813	2820	2798	2800	2797
% Crude protein	18.05	18.1	18.2	18.1	17.92	18.04	18.1	18.0	17.94	18.0
Lysine%	0.99	0.99	1.0	1.0	0.98	0.99	1.0	0.98	0.97	0.97
Methionine%	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Methionine+ Sistine%	0.69	0.69	0.70	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Calcium%	3.46	3.39	3.36	3.33	3.36	3.33	3.46	3.33	3.46	3.39
Available Phosphorus%	0.50	0.41	0.38	0.35	0.38	0.41	0.35	0.41	0.38	0.35
Arginine	0.85	0.85	0.86	0.86	0.85	0.85	0.85	0.85	0.83	0.83
Linoleic acid%	1.29	1.29	1.29	1.29	1.28	1.28	1.28	1.30	1.30	1.30

(1) T2 and T3 and T4 represent sources of phytase enzyme treatments fungal ' bacterial and mixture respectively
(2)Protease Center for poultry feed Breedcom-5 special product by Dutch WAFI company, (3) Metabolic Energy (kilocalories = 2100 crude protein 40%, fat 5%, crude fiber 2%, Calcium 8%, phosphorus 2%, lysine 3.75%, methionine 2.85%, of the date, and according to the (NRC, 1994)

Table 2. Components and chemical composition (%) of the diet used in laying hens from age 29 to 45 week

Treatment / Components	T1 control	T2,T3, T4(1)			(S.C)T5			T6(ALFALFA)		
		A FTY 250	B FTV 350	C FTY 450	A FTY 2.5	B FTY 3.5	C FTY 4.5	A FTY 2.5	B FTY 3.5	C FTY 4.5
Corn	50.74	50.43	50.72	50.517	50.334	50	50.009	51.087	51.85	52.777
wheat	18.34	19	18.15	18.983	19	19.49	19.8	17	16.3	15
SBM (44% cp)	17.3	17.35	18.06	17.57	17.3	17.221	17	17.2	16.662	16.59
Protein Centre (2)		5	5	5	5	5	5	5	5	5
fat	0	0	0	0	0	0	0	0	0	0
Di-calcium phosphate (18.5 P)	1.6	1.05	0.88	0.72	1.05	0.88	0.72	1.05	0.88	0.72
Calcium carbonate (limestone)	6.93	7.07	7.1	7.12	7.07	7.1	7.1	7.01	7.03	7.01
NaCl	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Yeast S.0	0	0	0	0	0.156	0.219	0.281	0	0	0
Dried leaves alfalfa	0	0	0	0	0	0	0	1363	2.188	2.813
Total	100	100	100	100	100	100	100	100	100	100
Calculated Chemical analysis(3)										
Energy (kcal I kg)	2722	2732	2733	2739	2731	2733	2739	2712	2712	2712
% Crude protein	16.4	16.5	16.7	16.5	16.5	16.5	16.5	16.4	16.3	16.3
Lysine%	0.85	0.86	0.87	0.86	0.86	0.86	0.86	0.85	0.84	0.84
Methionine%	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Methionine+ Sistine%	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.64
Calcium%	3.46	3.39	3.36	3.33	3.39	3.36	3.33	3.39	3.36	3.33
Available Phosphorus%	0.50	0.41	0.38	0.35	0.41	0.38	0.35	0.41	0.38	0.35
Arginine	0.85	0.85	0.86	0.86	0.85	0.85	0.85	0.85	0.83	0.83
Linoleic acid%	1.29	1.29	1.29	1.29	2731	2733	2739	1.30	1.30	1.30

Table 3. Components and chemical composition (%) of the diet used in laying hens from age 46 to 63 week

Treatment / components	T1 control	T2,T3, T4(1)			(S.C)T5			T6(ALFALFA)		
		A FTY 250	B FTV 350	C FTY 450	A FTY 2.5	B FTY 3.5	C FTY 4.5	A FTY 2.5	B FTY 3.5	C FTY 4.5
Corn	45.04	45.17	45.1	44.95	45.1	45.786	45.55	45.13	45.3	43.3
wheat	26.86	27.06	27.45	28.212	27.841	27	26.854	25.8	25	26.76
SBM (44% cp)	14	14.1	14	13.58	13.55	13.6	13.9	13.667	13.8	13.399
Protein Centre (2)	5	5	5	5	5	5	5	5	5	5
fat	0.2	0.18	0.1	0.07	0.05	0.05	- 0.05	0.4	0.442	0.65
Di-calcium phosphate (18.5P)	1.4	0.89	0.72	0.528	0.528	0.72	0.89	0.89	0.72	0.528
Calcium carbonate (limestone)	7.45	7.55	7.58	7.61	7.6	7.575	7.55	7.5	7.5	7.5
NaCI	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Yeast S.0	0	0	0	0	0.281	0.219	0.156	0	0	0
Dried leaves alfalfa	0	0	0	0	0	0	0	1.563	2.188	2.813
Total	100	100	100	100	100	100	100	100	100	100
Calculated Chemical analysis(3)										
Energy (kcal 1 kg)	2722	2733	2732	2737	2727	2733	2735	2723	2720	2127
% Crude protein	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6
Lysine%	0.78	0.78	0.78	0.77	0.78	0.78	0.78	0.77	0.78	0.77
Methionine%	0.37	0.36	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Methionine+ Sistine%	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.63	0.63	0.63
Calcium%	3.60	3.53	3.5	3.47	3.53	3.5	3.47	3.53	3.5	3.47
Available Phosphorus%	046	0.37	0.34	0.31	0.37	0.34	0.31	0.37	0.34	0.31
Arginine	0.77	0.77	0.78	0.78	0.77	0.77	0.77	0.77	0.77	0.77
Linoleic acid%	1.28	1.20	1.23	1.25	1.20	1.20	1.20	1.3	1.3	1.4

(1) T2 and T3 and T4 represent sources of phytase enzyme treatments fungal ' bacterial and mixture respectively (2)Protease Center for poultry feed Breedcom-5 special product by Dutch WAFI company, (3) Metabolic Energy (kilocalories = 2100 crude protein 40%, fat 5%, crude fiber 2%, Calcium 8%, phosphorus 2%, lysine 3.75%, methionine 2.85%, of the date, and according to the (NRC, 1994

For bacterial phytase we used *E. coli* sources of enzyme for fungal phytase *Aspergillus oryzae*, enzymes mixture contain (Xylanases, B-glucanase, Protease, Alfa-amylase, phytase, for yeast (*Saccharomyces cerevisia*), and for plant (dried alfalfa leaves powder).

Table 4. Estimation phytase enzyme in alfalfa leaves powder and *Saccharomyces cerevisia*

Material	Phytase enzyme activity (FTY/kg material)
Saccharomyces cerevisia	1600
Alfalfa plant leafs	160

The analysis was conducted in the Food Testing Center in Iowa in America.
It measured productive performance such as Hen day production (H.D.%) during 32 week for eight periods ,each period 28 days; cumulative egg production (egg/hen) during 224 day, egg weight, egg mass, feed conversions ratio, also qualitative characteristics of eggs such as yolk index, yolk weight, yolk and white relative weight and shell thickness.
Statistical analysis of this study using General, Linear Model of SAS statistical program was conducted (SAS, 2001).
To determine the effect of treatments studied it was using Complete Randomized Design.

RESULTS AND DISCUSSIONS

Table 5 shows no significant differences between treatments during the periods 1, 2, 3, 4, 5, 7 and 8 and the rate of egg production for the period 24-55wk recorded significant superior for treatments adding phytase enzyme at levels 250 and 450 FTY/kg feed and enzyme mixture at level 450 U/kg feed, so this was reflected positively to significant for the same treatments to produce cumulative eggs, followed by other treatments addition phytase enzyme than control group, while the lowest rate for this character recorded for the treatment added S.c.yeast at level (3.5/kg feed).
Table 6 revealed significant difference between treatments during all periods in egg weight, so egg weight from age 24-55 week has recorded higher rates for enzyme mixture at level 250 U/kg feed and alfalfa leaves powder at level 2.5 FTY/kg feed treatments by 65.45 and 65.30 gm respectively compared with enzyme treatments at various sources and levels (reduced with Ca, p) and control treatment which did not differ while adding yeast treatment at level 4.5 FTY/kg feed recorded the lowest rate of egg weight 62.65 gm.
Egg mass represents the number of eggs produced multiplied by the average egg weight and from the Table 7 there were no significant differences between experimental treatments during the first five periods and for the period 24-55 week treatment added fungal phytase enzyme at level(250 FTY/kg feed) recorded significant superior at rate 59.83 g/bird/day and a cumulative egg mass 13401 g/bird/224 day, while S.C. yeast treatment (3.5 FTY/kg feed) recorded minimum egg mass 54.32 gm/bird/day, which reflected negatively on the a cumulative egg mass 12167 g / bird / 224 day .
Feed consumption known indicator of the nutritional content that provided to birds and the limiting factor for the efficiency of production and profitability, especially that experimental diet adding phytase enzyme at different sources and levels low in Ca and P, and according to Lohman Guide provided 120 g feed / hen / day to meet the production need and did not recorded any residual amount of feed because all feed consumed from treatments birds .
Feed consumption is an important economic indicator and what determine the values of this index were feed conversion ratio and increase egg production, egg weight and other qualitative characteristics of eggs are only the result of inputs conversion efficiency to economic value outputs. Table 8 included the average feed conversion ratio for experimental treatments, there were significant differences (P <0.05) for all periods. When calculated rate of feed conversion ratio (g feed / g eggs) for a period of 24-55 weeks we showed significant improvement (P <0.05) for treatment of adding fungal phytase enzyme (250 FTY / kg feed) and treatment adding mixture enzymes with 2.01 and 2. 04 g feed / g egg respectively while S.C. yeast treatment (3.5 FTY / kg feed) recorded the worst feed conversion ratio (2.22 g feed/g eggs).
The relative weight of the shell, is an important indicator of shell thickness Table 9 revealed significant differences for all production periods between the various treatments in the experiment. The total rate of relative shell weight for period (24-55) leaves weeks, treatment of adding dried alfalfa leaf

powder at level (3.5 FTY / kg feed) showed significantly superior ($P<0.05$) recorded 10.39%, then fungal phytase enzyme treatment at level 250 FYT/ kg feed recorded 10.16% compared to other treatments.

These results showed a positive effect because the diets differed from the conventional, in terms of the fact that rations provided complete requirements of calcium and phosphorus for the bird while diets adding phytase enzyme various sources and levels (fungal, bacterial, a mixture of enzymes, S.c. yeast, and dried alfalfa leaves) reduced with Ca and P depend on phytase enzyme levels, and that hence the non-significant increase in these treatments compared with control group. The positive result in the search indicating the benefit for bird from unavailable phosphorus in the plant sources and its relationship with many nutrient and mineral linked with and reflection on the production, this may be due to the lytic action of phytase enzyme on phytic acid then release linked nutrients and mineral like proteins or carbohydrates or minerals and vitamins (Oatway et al., 2001; Gatlin et al., 2007; Hardy, 2010) ,the enzyme works to raise the level of phosphorus and availability and thus increase the performance of vital functions because of its role in metabolites of carbohydrates, amino acids, fat and entry in the synthesis of nucleic acids and storage of energy in the body (Cao et al., 2007). As well as the action of enzyme on the liberalization of calcium that enters in many processes and pathways of metabolic and increase the permeability of cell membranes, which helps the occurrence of absorption of nutrients in the intestines and facilitate fluid passage and some ions into and out of cells process and thus maintain the balance of the contents of (Fleet et al., 2008; Schoch et al., 2012), where each of Ca and P share together in many biological processes within the body and which affect directly to increase activity of thyroid gland as a result of secreted hormone responsible for regulating their levels in the blood, thereby increasing the metabolism which will reflect positively on egg production (Elsayed et al., 2010) in addition to release manganese by enzyme that important in activation many internal enzymes in the metabolism of protein, fat and carbohydrate to prowrement generated energy therefore is an important element in production of eggs (Yildiz et al., 2011). So release nutrient and mineral elements means increased availability and thus achieve maximum benefit from digested and absorbed (Peter and Baker, 2001; Aksakal and Bilal, 2002), in addition to release inositol to be benefit from body (Wu et al., 2006). This is another side to increase in egg production for treatments adding phytase enzyme at various sources and levels, particularly for the sixth period (44-47 weeks) of the production (Table 5) which coincided in the eighth month (August) of the year, which have very high temperatures (33-34.5ºC), that means exposure of birds to heat stress led to negative impact on the productive performance, but experimental treatments have proven that add phytase enzyme at various sources showed significantly superior ($P<0.05$) in comparison with control group (T1). This is due to the improvement in utilization of myo-Inositol which turns in the secondary path to vitamin C in animal body (Panda et al., 2007). On the other hand entry inositol in another path for the synthesis of fat in the body after decomposition glycerol several paths to get phosphotedil Inositol (Maurie et al., 2003) and therefore, both benefit of inositol whether to get vitamin C or release energy are important sources to reduce heat stress during this period, which reflected positively in improving productivity traits especially production eggs (Panda et al., 2008).

The superiority significant for the treatment of adding a mixture of enzymes at levels (350 and 450 u/kg feed) may be due to the enzyme mixture, which included Xylanases, ß-glucanase, α-Amylase, phytase, the Xylanases enzyme works on Arabinoxylase, and ß-glucanase, works on beta-glucane which connect many nutrients and thus reduce viscosity of intestine, also α-Amylase enzyme is working on analysis of starch and that some of it is linked to phytic acid after its release by phytase enzyme (Cowieson et al., 2008) that the mixture enzymes reflected positively on egg production.

The superiority shown for the treatments of adding dried alfalfa leaves powder (3.5 and 4.5 FTY/kg feed), which was similar to fungal phytase enzyme treatment at level (350 FTy/kg feed)in cumulative egg production during 224 days may be due to the contains of

alfalfa with many vitamins such as vitamin A, vitamin E and carotenoids (Mohammed and Al-Janabi, 1989) and because of its effect in increasing egg production in birds as a result of their impact on increased secretion of sex hormones that works as vitamin E to stimulate the secretion Luteinizing Hormone Releasing Factor (LHRH) from hypothalamus (Karanth et al., 2004), and thus enhance the secretion of both hormones FSH and LH and progesterone and estrogen, which leads to enhance egg production to the presence of a positive and significant between the concentration of these hormones in blood plasma and egg production.
I believe that the findings from the results when we added phytase enzyme with reduction in Ca and P lead to get maximum benefit from the nutrients release from feed and thus appeared clearly in many traits.
We find that the significant increase for treatments adding phytase enzyme at various sources and levels with decrease Ca and P in rations compared with control treatment (T1) led a positive reflection in the production process and this can be explained by several reasons, including that the most important factor in the impact on production is nutrition and especially the productive performance of laying hens is influenced by the level of the source of diets and in addition to other factors related to management of birds, this side reflects the superiority of treatments adding phytase enzyme at various sources and levels on egg weight to the ability of enzyme to analysis phytic acid and release nutrients either carbohydrates, and fats, and proteins, and elements to be more availability , thus affecting to increase the size of product egg (Liu et al., 2007), also release phytase enzyme for magnesium, which is an important element in metabolism of protein and protein synthesis (Maguire and Cowan, 2002), as is magnesium responsible for activated alkaline phosphatase enzyme (ALP) that being necessary for deposition of calcium and phosphorus in bones and increases metabolism of calcium and phosphorus, which encourage the transition of vital components of blood to liver, thus increasing liver function of vital components manufacturing (proteins, carbohydrates, fats) to go overage the need for management and production, transmission the necessary nutrients for the manufacture of egg yolk components (Weiser et al., 1990). Also the role of enzyme in release inositol ring which is part of secondary course be vitamin C (antioxidant), where he pointed by Panda et al., 2008, that have positive effect of the reaction of vitamin C on poultry production of eggs, weight and egg mass associated with increased protein concentration in egg yolk. And perhaps improve unsaturated fatty acids (Omega-3 and Omega-6). When we added phytase enzyme thus release associated fat and may have a role in improving chicken requirements from the essential fatty acid, in addition to advantage of vitamins soluble in fat (A, E, D, K), which is positively reflected a significant improvement to egg weight (Zyla et al., 2012).
Adding dried alfalfa leaves powder at level (2.5 FTy/kg feed) may be due to their contain with many enzymes such as phytase, amylase, beta-glucanase (Edimister et al., 2001), amylase works on analysis of starch into its basic components, and beta-glucanase which works to break down the compound complex beta- glucane and release nutrients associated with it, which has a high affinity for link with water, causing increase viscosity (Cowieson et al., 2006) and thus led to benefit from feed, which reflects positively on the production and egg weight. This is in addition to containing alfalfa a good quality protein and element such as calcium, phosphorus, and source of vitamin A.
The reason for increase relative shell weight in dried alfalfa leaves treatment may be due to contain alfalfa 9-13 gm/kg calcium is an important element in the formation of egg shell, as well as 2-3 gm/kg phosphorus, (Mohammed and Al-Janabi, 1989).Thus led to increase calcium by absorption from intestine and then move into blood and increase concentration of calcium in blood plasma (Al-Hassani, 2000), in addition to the containment. Flavonoids known as Isoflavones and glycoside saponine compounds for its role in influencing stimulation of sex hormones as estrogen that works to transform calcium from medullary bone to blood.
This reflected positively on increased shell thickness and shell weight, and this enhance the strength and hardness for egg produced (Fayad and Nagy, 1989).

Table 5. Effect of adding different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) in the perecentage of egg production (HD%) (±standard error) during production periods (24-55 weeks) of age

Treatments		Average percentage of egg production (HD%) of production periods (weeks)								Overall average 24 -55 weeks	Cumulative production of eggs (egg/chicken/224 day)
		1 27 −24	2 31 −28	3 35 −32	4 39−36	5 43 −40	6 47−44	7 51 −48	8 55−52		
T1		5.70±87.5	2.20±96.3	2.50±94.2	4.51±87.9	3.3±85.0	2.20±65.1 b	0.61±84.7	0.6±88.7	0.77± 86.26 cde	0.57± 193.21 f
T2	A	0.29±88.5	0.17±98.0	1.11±95.3	4.21±90.3	1.1±90.1	3.72±85.4 a	4.7±94.2	2.8±94.0	0.57±92.06 a	0.57±206.21 a
	B	0.89±78.5	1.92±88.7	2.01±88.5	7.52±92.7	8.8±91.4	6.92±86.4 a	2.9±88.0	3.9±90.4	0.50±88.13 bc	0.57±197.42 c
	C	1.42±88.2	0.12±94.3	0.31±95.0	5.62±91.9	4.4±88.1	4.40±86.6 a	4.2±90.2	2.4±92.3	2.10±90.87 a	0.57± 203.55 b
T3	A	7.81±82.7	2.20±92.2	3.50±90.7	3.50±85.9	4.2±87.2	5.32±84.6 a	6.0±90.2	4.1±87.7	0.75±87.72 bc	0.57±196.48 cd
	B	0.05±83.5	0.05±94.1	0.11±92.5	4.60±86.1	0.9±84.8	3.22±82.8 a	5.2±86.4	4.1±87.2	0.73±87.22 bc	0.57±195.36 de
	C	10.6±88.8	8.92±93.7	9.40±90.4	5.42±91.3	1.1±90.2	5.14±83.4 a	7.2±86.0	7.8±84.8	0.50±88.24 b	0.57±197.65 c
T4	A	3.80±83.5	2.42±94.3	1.50±95.0	0.11±88.3	1.2±84.3	0.95±77.2 ab	0.1±86.5	2.8±87.5	0.69±87.11 bc	0.57±195.13 de
	B	0.53±87.0	1.43±93.2	0.11±92.9	0.82±86.7	0.5±86.2	0.67±78.8 ab	2.6±85.1	0.2±85.4	0.48±86.98 bc	0.57 ±194.84 def
	C	6.01±86.3	1.32±96.7	1.2±96.41	5.50±90.6	1.6±92.9	6.10±84.3 a	2.1±93.6	1.5±90.3	0.59±91.85 a	0.57±205.75 a
T5	A	0.29±84.1	0.23±90.4	1.1±91.13	0.07±88.7	1.4±87.9	0.97±82.3 a	2.1±82.4	0.7±85.1	0.72±86.53 bcd	0.57± 193.83 ef
	B	2.92±81.6	0.35±91.5	1.9±91.10	0.71±85.7	1.1±83.5	1.67±73.3 ab	1.3±86.2	2.6±83.1	0.66±84.55 e	0.57± 189.40 g
	C	8.03±83.8	6.09±95.3	1.3±95.31	3.60±83.6	2.3±85.2	2.30±85.6 a	1.9±87.2	1.9±83.8	0.50±87.54 bc	0.57±196.08 cd
T6	A	4.92±78.9	7.40±89.7	8.3±90.32	10.0±88.0	6.0±86.6	6.01±82.4 a	1.3±83.2	9.3±81.7	0.55± 85.06 de	0.57±190.53 fg
	B	4.34±83.6	0.32±92.1	2.1±95.20	1.56±89.7	4.8±83.7	3.91±84.8 a	1.9±89.2	2.3±87.6	0.63± 88.29 b	0.57±197.77 c
	C	1.96±84.7	1.63±96.7	1.7±93.90	6.10±94.6	0.8±85.9	0.14±81.1 ab	1.9±83.6	3.8±81.3	1.01±87.77 bc	0.57±196.61 cd
significant		N.S	N.S	N.S	N.S	N.S	*	N.S	N.S	*	*

* Different letters within the same column indicated the presence of significant differences ($p \leq 0.05$),. T1= control. T2 and T3 and T4 enzyme phytase treatments fungal *Aspergillus oryzae* , bacterial E.coli and a mixture of enzymes, respectively ,and the letters A, B, C (250, 350, 450 FTY 1kg feed T5 and T6 treatment me yeast SC and alfalfa plant respectively, letters C, B, A (2.5 and 3.5 and 4.5 FTY /kg feed).

Table 6. Effect of adding different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) average of egg weight (g) (±standard error) during production periods (24-55 weeks) of age

Treatments		Average egg weight (g) of production periods (weeks)								Average egg weight (g) 24.55 weeks
		1 27 -24	2 31 -28	3 35 -32	4 39-36	5 43 -40	6 47-44	7 51 -48	8 55-52	
T1		0.05±64.01 ab	0.05±66.51 ab	0.01±65.06 ab	0.02±63.51 abc	0.03±61.07 ab	0.08±62.20 c	0.02±65.22 ab	0.08±65.92 b	0.07±64.30 abc
T2	A	0.02±64.65 ab	0.01±64.53 cde	0.63±64.80 ab	0.08±63.91 abc	0.44±61.83 ab	0.06±63.14 abc	0.09±65.55 ab	9.01±68.39 a	0.00±65.00 ab
	B	0.03±63.75 abc	0.06±65.36 abcde	0.01±64.38 abc	0.04±64.78 ab	0.55±62.85 ab	0.06±64.22 ab	b0.53±65.32 a	0.03±67.38 ab	0.05±64.75 abc
	C	0.08±63.85 abc	0.09±63.70 e	0.50±63.6 bc	0.05±63.10 c	0.04±60.23 b	0.02±62.91 abc	0.41±63.23 b	0.04±65.33 b	0.01±63.25 c
T3	A	0.05±63.65 abc	0.09±65.30 abcde	0.09±64.32 abc	0.60±65.24 ab	0.90±62.70 ab	0.96±63.52 abc	0.00±65.08 ab	0.21±66.85 ab	0.04±64.60 abc
	B	0.03±64.33 ab	0.06±66.63 a	0.08±65.64 a	0.07±64.50 ab	0.08±61.71 ab	080±63.21 abc	0.21±65.48 ab	2.01±65.72 b	0.10±64.70 abc
	C	0.05±64.25 ab	0.01±65.91 abc	9.01±64.89 ab	0.10±65.82 a	0.08±63.70 ab	0.45±64.17 ab	0.03±64.72 ab	0.03±66.86 ab	0.02±65.05 ab
T4	A	0.05±64.45 ab	0.00±66.72 a	0.09±65.59 ab	0.08±64.80 ab	0.06±62.80 ab	0.01±64.80 ab	0.02±67.20 a	0.43±67.39 ab	0.45±65.45 a
	B	0.03±63.32 abc	0.03±65.33 abcde	0.02±63.82 abc	0.99±63.92 abc	0.33±61.16 ab	0.02±65.62 a	0.54±65.50 ab	0.21±67.41 ab	0.40±64.53 abc
	C	0.02±63.75 abc	0.03±65.73 abcd	0.08±63.72 abc	0.66±63.92 abc	0.11±60.91 ab	0.04±63.24 abc	0.43±65.63 ab	0.05±66.21 b	0.55±64.15 abc
T5	A	0.02±63.76 abc	0.07±63.72 e	0.08±64.63 ab	0.06±63.31 abc	0.07±61.61 ab	0.21±63.34 abc	0.03±64.91 ab	0.01±63.94 b	0.05±63.65 b
	B	0.01±64.52 ab	0.05±64.82 bcde	0.03±65.13 ab	0.08±64.40 abc	0.07±62.40 ab	0.03±62.21 c	0.60±65.39 ab	1.02±64.81 b	0.06±64.20 abc
	C	0.04±62.20 c	0.04±63.68 e	0.04±62.53 c	0.87±63.75 ab	0.08±59.80 b	0.40±62.23 c	0.03±64.09 b	0.32±64.44 b	0.05±62.65 c
T6	A	0.06±65.05 a	0.08±66.80 a	0.08±65.38 ab	0.90±65.14 ab	0.04±63.34 ab	0.05±64.50 ab	0.03±65.19 ab	0.21±66.68 ab	0.20±65.30 a
	B	0.06±63.00 b	0.06±64.07 de	0.00±64.14 abc	0.09±64.51 ab	0.01±65.92 a	0.03±63.90 abc	0.02±64.80 ab	0.33±65.73 b	0.45±64.51 abc
	C	0.05±63.85 abc	0.01±66.43 ab	0.01±65.24 ab	0.60±65.17 ab	0.05±63.19 ab	0.20±64.20 ab	0.04±64.73 ab	0.11±65.92 b	0.03±64.85 ab
significant		*	*	*	*	*	*	*	*	*

* Different letters within the same column indicated the presence of significant differences ($p \leq 0.05$),. T1= control. T2 and T3 and T4 enzyme phytase treatments fungal *Aspergillus oryzae* , bacterial E.coli and a mixture of enzymes, respectively, and the letters A, B, C (250, 350, 450 FTY 1kg feed T5 and T6 treatment me yeast SC and alfalfa plant respectively, letters C, B, A (2.5 and 3.5 and 4.5 FTY /kg feed).

Table 7. Effect of adding different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) in egg mass (g /bird / day) (± standard error) during production periods (24-55 weeks) of age

Treatment		day) for production periods (weeks)/ bird/Average egg mass (g								Average from 24-55 weeks	Cumulative egg mass (g/ bird / day 224)
		1 27 -24	2 31 -28	3 35 -32	4 39-36	5 43 -40	6 47-44	7 51 -48	8 55-52		
T1		0.03±56.50	0.67±64.03	0.04±61.28	0.08±55.81	0.07±51.90	0.35±40.67 b	0.01±55.22	0.02±58.45 ab	±55.461.23 efgh	±124235.72 j
T2	A	0.02±57.21	0.01±63.25	0.23±61.75	0.34±57.70	0.05±55.68	0.01±53.92 a	0.03±61.70	0.04±64.28 a	±59.831.76 a	±134016.90 a
	B	0.03±50.04	0.05±57.97	0.00±55.97	0.09±60.05	0.30±57.39	0.03±55.46 a	0.06±57.46	0.02±60.91 ab	±57.071.09 cde	±127833.67 e
	C	0.01±56.31	0.03±60.06	0.03±60.42	0.87±57.98	0.34±53.03	0.05±54.47 a	0.03±57.00	0.05±60.29 ab	±57.502.13 bc	±128795.09 c
T3	A	0.70±52.63	0.12±60.20	0.09±58.32	0.12±56.04	0.05±54.67	0.06±53.72 a	0.07±58.70	0.04±58.62 ab	±56.671.26 cdefg	±126936.72 g
	B	0.05±53.69	0.02±62.67	0.11±60.68	0.34±55.53	0.09±52.33	0.02±52.32 ab	0.04±56.50	0.03±57.29 ab	±56.401.98 cdefg	±126339.02 h
	C	0.10±55.77	0.09±61.75	0.21±58.66	0.45±60.07	0.45±57.45	0.51±53.51 a	2.07±55.64	0.08±56.69 ab	±57.411.34 bcd	±128595.34 d
T4	A	0.38±53.81	0.03±62.89	0.34±62.31	0.45±57.21	0.04±52.94	0.06±50.02 ab	0.04±58.12	0.02±58.96 ab	±57.051.98 cde	12778±5.47 e
	B	0.50±55.12	0.13±60.85	0.40±59.28	0.09±55.40	0.06±52.71	0.02±51.69 ab	1.03±55.74	0.04±57.56 ab	±56.122.21 cdefgh	±125715.88 i
	C	0.06±55.01	0.22±63.53	0.06±61.40	0.72±57.89	0.05±56.57	0.04±53.27 a	0.12±61.40	0.03±61.56 ab	±58.922.01 ab	±131972.34 b
T5	A	0.29±53.57	0.11±57.58	0.13±58.89	0.43±56.14	0.06±54.14	0.05±52.12 ab	0.03±53.47	0.01±54.37 b	±55.091.40 fgh	±123396.87 k
	B	0.29±52.63	0.23±59.31	0.01±59.33	0.05±55.19	0.09±52.10	0.02±45.59 ab	0.04±56.36	0.03±53.84 b	±54.321.39 h	±121675.67 m
	C	0.08±52.12	0.10±60.68	0.15±59.59	0.32±51.74	0.04±50.94	0.04±53.24 a	0.06±55.88	0.02±53.96 b	±54.821.58 gh	±122807.83 l
T6	A	0.49±50.86	0.02±59.91	0.88±59.03	0.47±57.32	0.02±54.81	0.03±53.14 a	0.43±54.23	0.06±54.47 b	±55.531.03 defgh	±124397.71 j
	B	0.03±52.66	0.21±59.00	0.09±61.06	0.34±57.85	0.03±55.15	0.06±54.18 a	0.12±57.80	0.03±57.50 ab	±56.961.89 cdef	±127606.85 f
	C	0.19±54.08	0.42±64.23	0.12±61.26	0.04±61.65	0.05±54.28	0.05±52.06 ab	0.34±53.95	0.06±53.57 b	±56.922.01 cdef	±127495.50 f
significant		N.S	N.S	N.S	N.S	N.S	*	N.S	*	*	*

* Different letters within the same column indicated the presence of significant differences (p≤0.05),. T1= control. T2 and T3 and T4 enzyme phytase treatments fungal *Aspergillus oryzae* , bacterial E.coli and a mixture of enzymes, respectively ,and the letters A, B, C (250, 350, 450 FTY 1kg feed T5 and T6 treatment me yeast SC and alfalfa plant respectively, letters C, B, A (2.5 and 3.5 and 4.5 FTY /kg feed).

Table 8. Effect of adding different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) In feed conversion ratio (g feed / g egg) (± standard error) during production periods (24-55 weeks) of age

Treatment		Feed conversion ratio (g feed /g egg) for production periods (weeks)								Total feed conversion ratio (24-55) week
		1 27 -24	2 31 -28	3 35 -32	4 39-36	5 43 -40	6 47-44	7 51 -48	8 55-52	
T1		0.15±2.12 f	0.15±1.87 h	0.06±1.96 fg	0.09±2.15 b	0.07±2.31 b	0.13±2.94 a	0.25±2.17 b	0.01±2.05 de	0.00±2.20 ab
T2	A	0.05±2.10 f	0.02±1.90 gh	0.04±1.94 g	0.05±2.08 c	0.00±2.15 gh	0.03±2.22 efg	0.08±1.94 i	b0.06±1.79 h	0.03±2.01 f
	B	0.01±2.40 a	0.03±2.07 a	0.04±2.10 a	0.08±2.00 d	0.17±2.09 i	0.23±2.16 h	0.10±2.09 efg	0.14±1.97 f	0.17±2.11 de
	C	0.02±2.13 f	0.00±1.99 cd	0.04±1.98 ef	0.02±2.07 c	0.03±2.26 d	0.10±2.20 g	0.07±2.10 def	0.15±1.99 f	0.01±2.09 e
T3	A	0.15±2.28 c	0.13±1.99 cd	0.10±2.06 b	0.09±2.14 b	0.02±2.19 ef	0.06±2.23 efg	0.15±2.04 h	1.10±2.05 de	0.09±2.12 de
	B	0.01±2.23 d	0.03±1.91 fg	0.03±1.98 ef	0.13±2.16 b	0.07±2.29 bcd	0.06±2.29 d	0.08±2.12 cde	0.09±2.09 c	0.02±2.13 d
	C	0.15±2.17 e	0.00±1.94 ef	0.10±2.05 bc	0.02±2.00 d	0.00±2.09 i	0.09±2.24 ef	0.12±2.15 cb	0.07±2.11 c	0.05±2.09 e
T4	A	0.08±2.23 d	0.02±1.91 fg	0.05±1.93 g	0.09±2.10 c	0.56±2.26 d	0.02±2.39 c	0.05±2.06 gh	0.03±2.03 e	0.02±2.11 de
	B	0.12±2.18 e	0.01±1.97 de	0.05±2.02 cd	0.03±2.16 b	0.26±2.27 cd	0.11±2.32 d	0.08±2.15 bc	0.03±2.08 cd	0.06±2.14 cd
	C	0.01±2.18 e	0.04±1.89 gh	0.01±1.95 fg	0.01±2.08 c	0.13±2.12 hi	0.09±2.25 e	0.34±1.95 i	0.28±1.93 g	0.11±2.04 f
T5	A	0.09±2.24 d	0.00±2.08 a	0.02±2.04 bcd	0.09±2.14 b	0.00±2.21 e	0.16±2.30 d	0.66±2.24 a	0.08±2.21 ab	0.05±2.18 b
	B	0.06±2.28 c	0.01±2.02 bc	b0.01±2.02 cd	0.00±2.17 b	0. 04±2.30 bc	0.13±2.63 b	0.04±2.13 cd	0.06±2.22 ab	0.01±2.22 a
	C	0.19±2.30 c	0.09±1.98 d	0.12±2.01 de	0.03±2.32 a	0.09±2.35 a	0.03±2.25 e	0.00±2.15 bc	0.07±2.22 ab	0.06±2.20 ab
T6	A	0.02±2.36 b	0.08±2.00 bcd	0.20±2.03 bcd	0.05±2.09 c	0.03±2.19 e	0.10±2.25 e	0.11±2.21 a	0.07±2.20 b	0.09±2.17 bc
	B	0.06±2.28 c	0.06±2.03 b	0.03±1.96 fg	0.01±2.07 c	0.05±2.17 fg	0.05±2.21 fg	0.04±2.07 fgh	0.04±2.08 cd	0.08±2.11 de
	C	0.01±2.22 d	0.07±1.87 h	0.03±1.96 fg	0.05±1.95 e	0.10±2.21 e	0.01±2.30 d	0.05±2.22 a	0.11±2.24 a	0.06±2.12 de
significant		*	*	*	*	*	*	*	*	*

Table 9. Effect of addina different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) In relative shell weight(%) (± standard error) during production periods (24-55 weeks) of age

Treatment		**relativesheliweight for production Periods (week)**								**Rate the relative weight of the shell 24-56 weeks**
		1 24-27	2 28-31	3 32-35	4 36-39	5 40-43	6 44-47	7 48-51	8 52-55	
T1		035±9.39 c	0.35±9.76 abc	0.37±9.59 abc	0.35±9.45 abcd	0.0.37±8.73 ef	0.37±9.20 c	0.38±10.5 ab	0.32±10.00 cde	0.1419.58 bc
T2	A	0.37±10.27 bc	0.35±10.08 ab	0.3419.88 abc	0.35±9.48 abcd	0.32±9.99 abcd	0.34±10.14 abc	0.39±9.83 bc	0.37±11.61 a	0.16±10.16 ab
	B	0.37±9.36 bc	0.371,9.97 ab	0.36±9.60 abc	0.371-9.58 abcd	0.34±10.00 abcd	0.33±9.14 c	0.37±8.09 d	0.35±10.30 dc	0.17±9.54 bc
	C	0.33±9.51 c	0.361:10.29 ab	0.35±9.52 abc	0.37±9.59 abcd	0.34±8.98 cdef	0.32±9.45 bc	0.37±10.64 ab	0.38±9.18 de	0. 15±9.65 bc
T3	A	0.36±10.20 bc	0.34±8.74 c	0.34±9.51 abc	0.32±9.51 abcd	0.31±8.98 cdef	0.37±10.02 abc	0.38±9.04 cd	0.34±9.43 f	0.20±9.44 C
	B	0.37±10.27 bc	0.35±10.07 ab	0.33±7.34 e	0.33±10.57 a	0.34±8.11 f	0.361±10.00 abc	0.381±10.16 bc	0.37±9.28 f	0.27±9.48 c
	C	0.34±9.26 c	0.36±10.27 ab	0.36±10.36 a	0.34±9.22 bcd	0.34±9.41 bcde	0.34±10.59 ab	0.35±9.69 bc	0.35±9.14 f	0.20±9.74 bc
T4	A	0.37±9.41 c	0.34±8.72 c	0.33±8.78 cd	0.35±10.02 abc	0.31±10.77 a	0.31±9.54 bc	0.35±10.77 ab	0.36±9.07 e	010±9.63 bc
	B	0.361±9.80 bc	0.30±9.89 abc	0.32±10.40 a	0.33±10.05 ab	0.32±10.28 ab	0.33±10.28 abc	0.39±10.67 ab	0.39±9.39 f	0.19±10.10 abc
	C	0.35±10.73 ab	0.33±9.78 abc	0.3619.33 abc	0.32±9.80 abcd	0.35±8.89 def	0.36±9.69 abc	0.33±7.43 e	0.331±10.05 cde	0.19±9.45 c
T5	A	0.36±10.26 bc	0.32±9.76 abc	0.36±7.91 de	0.34±9.29 bcd	0.30±9.84 abcd	0.37±10.80 a	0.31±10.65 ab	0.36±10.16 cde	0.31±9.84 abc
	B	0.34±9.76 bc	0.36±9.23 bc	0.33±9.51 abc	0.35±9.53 abcd	0.36±9.42 bcde	0.31±9.46 bc	0.33±10.32 ab	0.34±9.56 cde	0.2029.60 bc
	C	0.36±9.74 bc	0.35±7.59 c	0.33±10.36 a	0.33±9.45 abcd	0.34±10.13 abc	0.33±9.57 bc	0.36±9.84 bc	0.34±10.63 abc	0.12±9.66 bc
T6	A	0.36±9.36 c	0.32±10.57 a	0.38±8.99 bc	0.27±8.69 d	0.33±10.46 ab	0.30±10.00 abc	0.34±9.85 bc	0.33±10.00 cde	0.23±9.74 bc
	B	0.34±10.47 abc	0.38±10.13 ab	0.32±9.99 ab	0.33±8.84 cd	0.32±9.87 abcd	0.30±9.87 abc	0.36±11.47 a	0.38±11.47 ab	0.16±10.39 a
	C	0.34±11.50 a	0.33±10.09 ab	0.34±10.31 a	0.33±9.05 bcd	0.35±7.92 f	0.31±6.92 d	0.34±9.69 bc	0.34±10.47 bc	0.21±9.49 c
significant		*	*	*	*	*	*	*	*	*

* Different letters within the same column indicated the presence of significant differences ($p \leq 0.05$),. T1= control. T2 and T3 and T4 enzyme phytase treatments fungal *Aspergillus oryzae* , bacterial E.coli and a mixture of enzymes, respectively ,and the letters A, B, C (250, 350, 450 FTY 1kg feed T5 and T6 treatment me yeast SC and alfalfa plant respectively, letters C, B, A (2.5 and 3.5 and 4.5 FTY /kg feed).

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