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THE EFFECT OF ADDING PHYTASE AND PROTEASE ENZYMES AND THEIR MIXTURE TO THE DIET ON THE PRODUCTION PERFORMANCE OF BROILER

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Abstract

The experiment was conducted in a poultry field in Wasit province / Badra district for the period from 18/11/2019 to 29/2/2019. The aim of this study was to investigate the effect of adding phytase and protease enzymes to the broiler diets on productive performance and some physiological traits, using 400 one day old female chicks of broiler of (Ross 308). The study included 4 treatments, each treatment included four replicates of 25 chicks / rep, and chicks were randomly distributed on the experiment treatments as follows: The first treatment T_1 was control treatment, the birds were fed with a diet free of additives and without any reduction of Ca and P levels, treatment T_2 birds were fed with a diet containing 500 FTY phytase enzyme / 100 kg feed, and reduce the Ca and P levels in the diet. Treatment T_3 birds were fed with a diet containing 500 FTY phytase enzyme and 250 FTY protease enzyme / 100 kg feed and without any reduction of Ca and P levels. Finally treatment T_4 birds were fed with a diet containing 500 FTY phytase enzyme and 250 FTY of the protease / 100 kg feed, by a reduction of 0.33% and 0.15%. For Ca and Phosphorus, respectively. The results showed that there was a significant increase in body weight, weight gain and feed consumption for the fourth treatment, which used phytase and protease enzymes with the reducing levels of P and Ca in the diet compared with the rest of the experiment treatments. There was a significant improvement at the level of ($P < 0.05$) in the food conversion factor for the second and fourth treatments, and the mortality of the fourth treatment decreased during the total period of the experiment compared to the rest of the treatments.

Key words : Phytase, Protease, Broiler.

Introduction

Feed additives are the best way to obtain the best productivity, where nutrition scientists have shown its great role in developing the poultry industry and achieving the economic feasibility from the point of view of the investors. There is another opinion of the ecologists that stipulating the development should not be at the expense of the environment, therefore, the researchers' has focused on both sides. (Rutz *et al.,* 2005). Enzymes are food additives that contribute to increase in availability of many essential nutrients such as proteins, fats and carbohydrates in addition to minerals and vitamins found in the diet, and increases its absorption ability into the digestive tract without the need to add some elements that are high in cost, in addition to reducing the disposal of some environmentally polluting substances with waste (Alam *et al.,* 2003). One of these enzymes is the phytase enzyme, which its efficiency is shown in hydrolysis phytic acid under suitable conditions. It represents the form in which phosphorus is found in the plant sources used in the bird's diets, where two-thirds of phosphorus is not available and associated with protein and several mineral elements and this enzyme is called as an environmentally friendly product (Wodzinski and Ullah, 1996) because it reduces the need to add phosphorus to the diet and reduces its releasing with glaucoma and thus reduces the environmental pollution (Parsons, 2005). World production of grain used for feeding poultry and farm animals contains about 14-16 million tons of phytate (Lott *et al.,* 2000; Peter and Sell, 2007). The advantage of P phytate in monogastric animals is limited to (40%) due to the lack of internal phytase enzyme required for disposal it (Wang *et al.,* 2017), and most of these complexes are

dissolved in the acidic environment (pH <3.5) of the proventriculus and gizzard (Walk *et al.,* 2013), The correlation with positively charged cations are strong and associated with greater force with the gradually increases of pH along the digestive tract (Williams, 2014), while phytate is the compound produced by the correlation of the phytate acid with potassium ions, magnesium and lower percentage of calcium (Maenz, Khalid 2001; *et al.,* 2013). The phytate is concentrated in the aleurone layer in the monocotyledons seed (wheat, barley and rice), while in the maize, it is concentrated in the endosperm (Hid vegi and Laztity, 2002). Phosphorus and minerals associated with the phytate are not absorbed and disposed with wastes. Thus, the diets must be supplemented by the additional quantities of it, which increases the cost of production (Nahm, 2007). Recent studies have shown that the addition of the protease enzyme in the diet increases the protein and amino acid digestion and improves the productive performance of broiler, which dependent in feeding on grains such as corn, wheat and soya beans (Angel *et al.,* 2011; Freita, *et al.,* 2011; Frungi *et al.,* 2014). Therefore, in recent years, it has been used as feed supplements to the diets to increase the digestion of protein found in the diet by the animal. It has been found that the protease enzyme works on the implementation of a wide range of complex physiological functions and it is very important in the metabolic processes and regulatory functions that occur in all forms of living organisms. It plays an important role in many physiological and pathological processes that occur within the body, such as the process of destroying vital effective proteins and production of amino acids needed by the body, as well as, its creation of enzymes, hormones, and peptides that bioactive from inactive compounds (Mala et al, 1998). Therefore, the current study aims to identify the effect of the enzymatic addition of phytase and protease in the production performance body weight, conversion factor, weight gain feed consumption ratio, and mortality percentage of broiler chicks.

Materials and Methods

The experiment was conducted in a poultry field in Wasit province / Badra district for the period of 18/11/ 2019 to 29/2/2019, the aim of this study was to know the effect of adding phytase and protease bacterial enzymes to broiler diets on productive performance and some physiological traits.

Experiment Design and Treatments

A 400 chicks from Ross-308 at one day old were used in this study, with an initial weight of 45 g / bird, the experiment lasted for 42 days, while the chicks were bought from the local hatchery in Wasit province, and were randomly distributed on 4 treatments. Each treatment contained four replicates, and each replicate contained on (25) chicks / rep divided into 16 Pen with a dimension of 2 x 3 m (length x width). The temperature was regulated within the required limits to obtain a temperature of 34°C during the first three days, then reduced to 32°C at the end of first week, and reduced by 2°C per week until it reached 24°C. Finally, Feed and water was provided freely throughout the experiment period. Continuous light method was used in the first seven days of age, then reduced to 20 hours / day with 2 hours break every 12 hours (5-6) using an electronic clock from the eight days to three days before the end of the experiment.

Experiment treatments

T_1: Control diet (without any addition and reduction).

T_2: a diet added to it phytase enzyme at a level of 500 FTU / Kg feed, by a reduction of 0.33% and 0.15%. For Ca and Phosphorus, respectively.

T_3: a diet added to it phytase enzyme at a level of 500 FTU / kg feed and protease enzyme at a level of 250 FTU / kg feed, without calcium and phosphorus reduction.

T_4: a diet added to it phytase enzyme at a level of 500 FTU / kg feed and protease enzyme at a level of 250 mg / kg feed, by a reduction of Ca 0.33% and 0.15%. For Ca and Phosphorus, respectively.

Nutritional diets

The diets were supplied from the feed factory (Feedco Company) in Erbil according to the special requirements of the Ross308 breed manual, and it was prepared according to the age stages during the experiment period and the starter diet was from 1-10 days, the growth diet from 11-21 days and the finisher diet from 22-42 days. Table 1 shows the composition of the diets used in the experiment.

Preventive program

The preventive program and health care were used as shown in table 2, as the chicks were thirsted for 4 hours before vaccines are provided with drinking water.

The source of enzyme used in the experiment

The bacterial phytase enzyme was obtained from Dretim Tesist Company /Turkey, the trade name FARMAZYME PHYTASE2400. This enzyme is produced by Butti auxella bacteria and manufactured in the form of fine spherical granules. This product is characterized by thermal stability up to 95 °C, and this trait is very important during the manufacturing process

of the pellets, and this enzyme has a relatively high efficiency in the low PH (2.5 - 5.5). While the bacterial protease enzyme was obtained from (AMERICAN) company/Amman, the trade name PIOSYSTEMS, this enzyme is produced by Bacillus spp bacteria and manufactured in the form of fine spherical granules. This product is characterized by thermal stability up to 90 °C, and this trait is very important during the process of manufacturing the pellets, and this enzyme has a relatively high efficiency in the PH ranges between (3.5 - 0.5).

The studied Productive traits studied

Average body weight and weight gain

The chicks were weighed for each replicate at the age of one day by a sensitive electronic balance after removing the feeder before 2-3 hours from the date of weight, while the average body weight was calculated through the following equation:

Average body weight (g) =

$$\frac{\text{Total birds weights (g)}}{\text{Number of birds}}$$

Moreover, the weight gain was calculated as follows: Weight gain (g) = body weight at the end of the period (g) - body weight at the beginning of the period (g).

Average feed consumption per week

The amount of feed consumed was calculated according to the following equation). The amount of feed consumed = the amount of feed provided at the beginning of the period (g) - the amount of feed remaining at the end of the period, and in case of mortality, the following equation was applied:

Daily consumed feed per bird =

$$\frac{\text{The amount of feed consumed for the period}}{\text{Number of birds}}$$

Food conversion factor

The food conversion factor was calculated according to the follows equation

Food conversion= factor (g. feed/ (g. weight gain))

$$\frac{\text{amount of feed consumed}}{\text{(total life birds weights + total weights of mortality birds) - total weights at the beginning of the period}}$$

Mortality percentage

The mortality was recorded, weighed and calculated according to the following equation:

Mortality percentage % =

$$\frac{\text{Total dead birds during the period}}{\text{Total number of birds at the beginning of the period}}$$

Production Index (PI)

The production index was calculated by applying the 20 equation as follows.

Production index scale =

$$\frac{\text{Average body weight (g)} \times \text{vitality ratio}}{\text{Number of reearding days} \times \text{food conversion factor } x\,10}$$

In which,

Vitality ratio = 100 - mortality percentage

Results and Discussion

Body weight and Weight gain (g)

Table 3 shows the effect of adding phytase and protease enzymes results on the average cumulative weekly body weight (1 - 42 days). It was observed that there are no significant differences in the first and third week, while the fourth treatment was significantly superior over the first and third treatments in the second week, while it did not differ significantly from the second treatment, which in turn did not differ significantly from the first and third treatments. During the fourth week, the fourth treatment recorded the highest rate of body weight compared to the first and second treatments, while the third treatment did not differ significantly from the first and second treatments, and did not differ significantly from the fourth treatment. During the fifth week, the third and fourth treatments were significantly superior over the control treatment, and the fourth treatment was superior over the second treatment. While there were no significant differences between the first and second treatments and between the second and third treatments and between the third and fourth treatments. In the sixth week of bird life, the fourth treatment was significantly superior to other treatments and recorded the highest average body weight at age 42 days (2297.063 g) compared to other treatments that showed no significant differences with each other. As for the weight gain, the results of Table 4 were observed that all the treatments was significantly increased in the weight gain for the period (1 - 42 days), and observed that there are no significant differences in the average weight gain during the first and third weeks of age. While in the second week, the fourth treatment was significantly superior over the first and third treatments. Also, the second treatment was superior over the first treatment, while there were no significant differences between the first and third

Table 1: Feed ingredients and calculated chemical composition of the diet used in the experiment.

Feed material	Starter diet 1-10 day		Growth diet 11-21 day		Finisher diet 22-42 day	
	PC	NC	PC	NC	PC	NC
Yellow Maize	57.12	58.78	61.92	62.55	64.15	64.8
Soybean*	35.55	35.45	30.7	30.6	28.2	28.1
Minerals and vitamins mixture**	1	1	1	1	1	1
Sunflower oil	1.55	1.35	2.95	2.75	3.5	3.3
$CaHPo_4$	1.8	0.85	1.6	0.67	1.4	0.5
limestone	1.2	1.70	1.00	1.65	1.00	1.60
Methionine	0.24	0.23	0.23	0.23	0.18	0.18
Lysine	0.24	0.24	0.25	0.25	0.17	0.17
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100	100
Calculated chemical composition according to NRC 1994***						
Crude protein. CP%	22	22	20	20	19	19
Metabolism energy ME	2954	2956	3089	3090	3152	3153
Meth + Cys	1.04	1.04	0.98	0.98	0.91	0.91
Lys%	1.39	1.39	1.26	1.26	1.13	1.13
Ca%	1	1	0.88	0.88	0.84	0.84
aP%	0.45	0.28	0.41	0.24	0.37	0.2

* Soybean meal used from an Argentine source, the percentage of crude protein was 48%, and the metabolism energy was 2440 k cal / kg.

** Mixture of minerals and vitamins used in the manufacture of diets Broiler Premix 1% produced by the Belgian company Intraco LTD free of calcium and phosphorus.

*** Chemical composition of the remaining feedstock based on NRC (1994).

Table 2: The preventive program and health care used in the experiment.

Vaccine and preventive measures	Age in days
Bivalent oil Vaccine Infections bronchitis and Newcastle disease	1
Spray Newcastle vaccine colon 30	1
Enzo floxacin antibiotic for 3 days	1
Newcastle vaccine (lasota strain) with drinking water+ Multivitamin	8
Gumboro vaccine (Intermediate strain) with drinking water	12
Newcastle vaccine (lasota strain) with drinking water	18
Enrol antibiotic	21
Newcastle vaccine (lasota strain)with drinking water	28
Tylosin	30

treatments and between the second and third treatments and between the second and fourth treatments. In the fourth and sixth week of age, the fourth treatment was significantly superior to the rest of the treatments, but in the fifth week, the third and fifth treatments recorded the highest weight gain compared to the control and a second treatment. In the total weight gain, the treatment recorded the highest weight gain followed by the third treatment compared to the first and second treatments.

Feed consumption ratio (g)

Table 5 showed the effect of the experimental treatments on the feed consumption ratio (gm feed/ g live weight) during the experiment weeks (1 - 42 days) as it is observed from the result, that there were no significant differences between the treatments in the first, third, fourth and fifth weeks of age, but in the second week, the fourth treatment was significantly superior over the first and third treatments as well as the second treatment was significantly superior over the first treatment, while there was no significant differences between the first and third treatments and between the second and third treatments and between the third and fourth treatments. As for the total feed consumption for the period 1-42 days, the third and fourth treatments were significantly superior over the first and second treatments.

Food conversion factor

Table 6 showed that there are no significant differences between the treatments in the first, second and sixth weeks of age, but in the third week there was a significant improvement in the fourth treatment compared with the first and second treatments while the third treatment did not differ significantly from the first and second treatments on the one hand and the fourth treatment on the other hand. At the fourth week of age, significant improvement was observed in the fourth treatment compared to the first and third treatments, while the second treatment was not significantly different from the first and third treatments and from the fourth treatment.

Mortality percentage

Fig. 1 shows significant differences in the mortality percentage, where it was observed that there is a significant decrease in the fourth treatment of mortality percentage compared with the other treatments.

Production Index

The results of the study in the Fig. 2 shown a significant superiority in the production index at the age of 6 weeks for the fourth treatment (275.3) compared to the control treatment and the latter did not record any

Table 3: Effect of Adding phytase and protease enzymes to the broiler diet on average body weight (g) for 1 - 42 Days.

Treatments / Weeks	Body weight (g)				LSD
	T_1	T_2	T_3	T_4	
Week 1	127.22±0.823	127.72±0.853	127.55±0.913	128.27±0.409	N.S
Week 2	323.27 ±3.758 b	335.07±2.978 ab	327.47±6.218 b	344.20±1.366 a	*
Week 3	624.62 ±5.818	627.37±4.389	635.87 ±3.953	641.62± 22.973	N.S
Week 4	1010.88 ± 7.012 b	1002.23±8.943 b	1028.70 ±2.905 ab	1055.69±25.062 a	*
Week 5	1530.29± 3.304 c	1548.69±14.283 bc	1580.05±2.6501 ab	1612.40±21.267 a	*
Week 6	2127.74 ±10.654 b	2156.74± 20.871 b	2173.10±45.598 b	2297.06±32.340 a	*

T_1: the control diet without any addition.

T_2: a diet added to it phytase enzyme at a level of 500 FTU / kg feed with a Ca reduced by 0.33% and phosphorus by 0.15%.

T_3: a diet added to it phytase enzyme at a level of 500 FTU / kg feed and protease enzyme at a level of 250 FTU / kg feed without calcium and phosphorus reduction.

T_4: a diet added to it phytase enzyme at a level of 500 FTU / kg feed and protease enzyme at a level of 250 mg / kg feed and with calcium reductions of 0.33% and phosphorus 0.15%.

* Indicates the presence of significant differences on a significance level of ($P< 0.05$)

N.S: indicates no significant differences.

Table 4: Effect of Adding phytase and protease enzymes to the broiler diet on average weight gain (g) for 1 - 42 Days.

Treatments / Weeks	Average weight gain (g)				LSD
	T_1	T_2	T_3	T_4	
Week 1	84.82 ± 0.905	84.72±1.086	84.55±1.239	85.27±0.523	N.S
Week 2	196.05 ± 3.372 c	207.35±2.336 ab	199.92±5.331 bc	215.93 ±1.039 a	*
Week 3	301.35 ± 2.420	292.3±6.0529	308.4±4.385	297.42±24.177	N.S
Week 4	386.26±8.599 b	374.86±6.8556 b	392.83±3.726 b	414.07±3.769 a	*
Week 5	519.41 ±11.012 b	546.46 ±7.983 ab	551.35±0.590 a	556.71±3.936 a	*
Week 6	597.45±47.718 b	608.05±23.403 b	593.05±9.083 b	684.66 ±17.837 a	*
Total weight gain	2084.74±55.118c	2013.74±41.384c	2130.1±41.384b	2254.06±41.384a	*

significant differences with the rest of the treatments that were reached (233.5, 233.5), respectively.

The improvement in the production traits of broiler in the above Tables may be due to the enzymatic activity of the phytase and protease, which increase the efficiency of releasing phosphorus and thus its availability to take advantage of it within the bird's body. (Kies *et al.*, 2001) and (Peter & Baker 2001) explained that the phosphorus level increased and its availability of the body when the diet contained the enzyme of the phytase that released phosphorus and other minerals associated with the phytate in the plant feed materials, especially yellow maize, and these minerals include calcium, magnesium, zinc and manganese, thus increasing the availability of these mineral elements, as well as, phosphorus and increasing its availability for fly and its absorption ability.(Akyurek *et al.*, 2005) pointed out that the ability of the phytase enzyme to release 35-60% of unavailable phosphorus, this increase may be the reason for increasing enzymatic interactions and metabolic activities, and this can stimulate feed and water intake as a result of stimulating appetite centers in the central nervous system and thus reflected on increased the body weight (Moshad, 2001; Baruah *et al.*, 2004; Boyce *et al.*, 2004). Furthermore, the phytase enzyme releases the protein associated with phytate as a result of partial destruction of plant cell walls involved in the diet composition, thereby increasing its exposure to various internal and external enzymes and increasing the body's advantage of them (Naher, 2002), not only the benefit of protein, but in releasing carbohydrate and fat from phytate complexes thus, energy levels rise and increased the benefit of inositol after hydrolysis the phytate by the phytase enzyme (Wu *et al.*, 2006). This is where the role of protease enzyme that analyzes proteins into amino acids and increases their absorption in the intestines (Giri and Rostami, 2013). This shows the role of the phytase and protease enzymes in improving the process of benefit from the nutrients associated with phytate and its reflection on increasing body weight. The increase in

Table 5: Effect of Adding phytase and protease enzymes to the broiler diet on feed consumption ratio for 1 -42 Days.

Treatments / Weeks	Feed consumption factor (g)				LSD
	T_1	T_2	T_3	T_4	
Week 1	104.87±1.473	105.92±1.427	105.17±1.297	106.05±1.246	N.S
Week 2	257.67±2.876 c	272.02 ±2.523 ab	260.65±6.409bc	280.87±1.5321 a	*
Week 3	439.55±3.179	421.00±8.391	441.95±12.637	412.27±33.691	N.S
Week 4	640.65±15.561	643.50±11.155	665.40±5.726	662.17±9.462	N.S
Week 5	992.50±18.753	995.55±14.819	1012.37±5.034	1007.68±9.414	N.S
Week 6	1291.79±13.468b	1323.17±24.668b	1315.29±13.857b	1348.28±8.403 a	*
Total feed consumed	3727.03±33.492b	3761.16±42.372b	3800.83±39.220a	3817.32±61.323a	*

Table 6: Effect of Adding phytase and protease enzymes to the broiler diet on food conversion efficiency during the experiment weeks.

Treatments / Weeks	Food conversion factor				LSD
	T_1	T_2	T_3	T_4	
Week 1	1.245±0.014	1.250±0.014	1.243±0.006	1.243±.0.013	N.S
Week 2	1.314±0.008	1.311±0.009	1.303±0.003	1.300±0.006	N.S
Week 3	1.458±0.0176 a	1.440±.008 a	1.433±0.022 ab	1.386±.015 b	*
Week 4	1.658±0.022 a	1.716±0.018 ab	1.693±0.021 a	1.599±0.036 b	*
Week 5	1.910±0.014 a	1.821±0.019 ab	1.836±0.008 b	1.810±.0082 b	*
Week 6	2.162 ±0.211	2.176±0.085	2.217±.046	1.969±.0581	N.S
Cumulative conversion factor	1.624±0.348a	1.619±0.492a	1.620±0.511a	1.551±0.359b	*

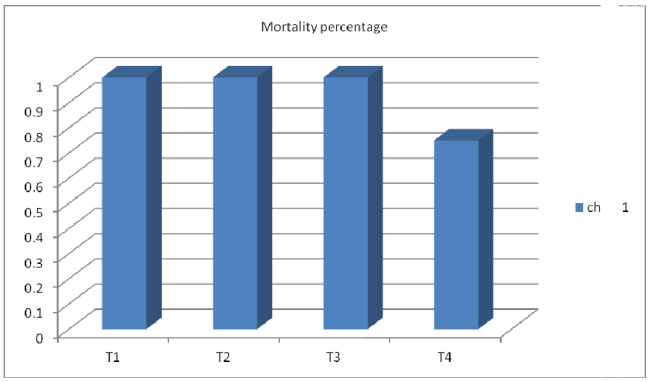

Fig. 1: Effect of phytase and protease enzymes on the mortality percentage % for the period (1 - 42 days).

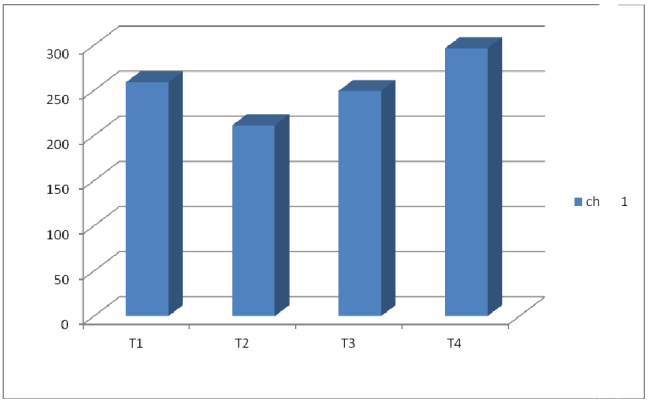

Fig. 2: Effect of phytase and protease enzymes on the production index.

feed consumption may be due to the effectiveness of adding an enzymatic mixture of phytase and protease to broiler diets from the first day and the fact that digestive juices are incomplete, the enzymatic addition increases with the aging of the bird this may lead to the expansion of the digestive tract, and the increase in the amount of feed consumption as (Cowieson and Adeola, 2005) pointed out. Both the microbial phytase and phosphide enzymes make a benefit of food, and also the phytase enzyme destroys the plant cell walls in the feed material.

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