



BIO-EFFICACY AND THERMO-STABILITY EVALUATION OF DIFFERENT PHYTASE ENZYMES IN PELLETTED AND MASH BROILER DIETS

Amira M. Refaie; Abdallah, A.G.; Nadia, L. Radwan; Abeer R. Khosht, Abdel-Khalek, A.M.

Anim. Prod. Res. Institut., ARC, Dokki, Giza, Egypt

Corresponding author: Amira M. Refaie e-mail : amira_refaie2@yahoo.com

Received:10/05/2016

Accepted: 29/05/2016

ABSTRACT:A total of 780 unsexed day old Arbor Acres chicks were used. The birds were randomly distributed into 26 treatments of 30 chicks each. Two studies were designed to compare three phytase sources differed in their microbial origin and thermo-stability on performance and bone mineralization of broilers. In Exp. 1, with less 40% available phosphorus (aP) diets than recommendation, chicks were fed 0.3% and 0.27% aP (-40% aP), during starter and grower periods, respectively, and supplemented with one of the following phytases; Phytase I (*Schizosaccharomyces pombe*), Phytase II (*Trichoderma reesei*) and Phytase III (*Pichia pastoris*) at 3 levels (0, 500 or 750 FTU/kg diet) each within two feed forms (mash or pellet) in 3×3×2 factorial design. In a concurrent study, with less 20 or 40% aP with the most stable phytase source from Exp. 1 at 0, 500 and 750 FTU/kg diet, also in mash or pellet feeds (Exp. 2) in 2×3×2 factorial arrangement. In both experiments, two control groups fed strain recommendation of aP (contained 0.5%, 0.45% aP during starter and grower periods, respectively) with the same feed forms were used for comparison. Results obtained indicate that:

Experiment 1: There is a difference in thermo-stability among phytase sources according to the microbial origin.

1- Chicks fed -40% aP and supplemented with either Phytase I or Phytase III had significantly higher weight gain and carcass percentage than Phytase II.

2- Chicks fed pellet diets recorded ($P \leq 0.05$) better growth performance compared to those on mash diets.

It could be concluded that: phytase I (*Schizosaccharomyces pombe*) is the most stable phytase, while, chicks fed -40% aP, irrespective of phytase source didn't reach the values of control group (strain recommendation of aP) concerning growth performance parameters or bone measurements.

Experiment 2: Chicks fed -20% aP diet improved feed conversion ratio (FCR) by 4.1% for overall period and higher plasma P and Ca by 34.8% and 27.7%, respectively comparing to others fed -40% aP.

1- Chicks fed dietary Phytase I at levels of 500 and 750 FTU/kg diet improved significantly live body weight, carcass % and better overall FCR.

2- By feeding pellet diet, chicks recorded better overall FCR by 4.1% than those fed mash diet. It could be recommended that addition of coated Phytase I (*Schizosaccharomyces pombe*) at 500 FTU/kg diet to -20% aP diet improved growth, bone mineralization of broiler chicks fed either mash or pellet diets.

Key Words: Broilers- Pellet diet- Mash diet- Thermo-stable phytase- Growth performance

INTRODUCTION

Sixty to seventy percent of phosphorus (P) in feedstuffs of plant origin, which form the bulk of poultry diets, is phytate-bound (Bedford, 2000). This phytate-bound P is poorly hydrolyzed by poultry because they do not produce sufficient amount of phytase enzyme that hydrolyzes phytate (Ravindran et al., 1995 ; Bedford, 2000). Thus, inorganic sources of P, which are expensive, are added in diets to meet P requirements of the poultry, leading to increased cost of feeding (Selle and Ravindran, 2007). Furthermore, phytate can bind positively charged nutrient in the gut, leading to their reduced availability for utilization by poultry as it (phytate) is negatively charged at all pH conditions found in the gut (Lenis and Jongbloed, 1999).

In recent years there has been a considerable interest in the use of microbial phytase to release phytate-bound P and to improve overall P availability in poultry diets. The following results demonstrate that supplemental phytase is effective in improving the availability of phytate-bound P for growth, bone mineralization, metabolizable energy and nutrient digestibility and nutrient retention in broiler diets (Ravindran et al., 2000; Zyla et al., 2001). Commercially, most of the feed used in poultry is consumed in the pelleted form. That implies that during the manufacturing process the meal has been subjected to temperatures usually in excess of 80°C, through direct exposure to steam. However, the phytase molecule has a limited thermal stability and studies have demonstrated that losses in activity begin to occur at around 60 °C (Ullah and Mullaney, 1996).

Phytase may be applied to a diet after pelleting either as granules or sprayed on as a liquid to avoid its loss of activity that is associated with pelleting (Selle and Ravindran, 2007). However, this method of phytase application requires heavy investment in the application equipment, and may result in non- uniform distribution

of phytase in the diet (Slominski et al., 2007). Coating of enzymes with a layer of fat or carbohydrates is widely used to improve thermo stability; however, different coating technologies may provide better protection than others Sulabo et al., (2011). Kirkpinar and Basmacioğlu (2006) concluded that the best pelleting temperature was 65°C while, they noted that feeding broiler chicks with pellet diet at temperature of 85°C damages phytase activity. On the other hand, Woyengo et al., (2010) reported that coated phytase (derived from *E. coli*) improved nutrient utilization in broilers, and its bio-efficacy was unaffected by pelleting process. Also, Wilkinson et al., (2013) compared two phytases different in microbes produced them (*Trichoderma reesei* or *Pichia pastoris*) on broilers performance and bone mineralization. They concluded that supplementation of phytase derived from *Trichoderma reesei* to broiler diet enhanced their performance and tibia ash also, improved thermo tolerance than others fed phytase (*Pichia pastoris*) supplemented diet. In addition to, dos Santos et al., (2013) who declared that using of high dosages of phytase (up to 1500 FTU/kg diet) significantly improved broilers FCR beyond that achievable with standard diets through the elimination of the anti-nutrient effects of phytate.

The aim of this study was to compare three phytase enzyme sources differed in their microbial origin and thermostability during pelleting process on performance and bone mineralization of broilers fed low available phosphorus diets (Experiment 1), while, evaluate the most stable phytase source on broiler performance and bone mineralization fed various levels of available phosphorus (Experiment 2).

MATERIALS AND METHODS

The experimental work was carried out at El-Fayoum poultry farm, Animal

Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt.

Phytases: Phytase I is sourced from an *E.coli* species bacterium and is expressed in a *Schizosaccharomyces pombe*. It is produced by company A, UK as a 6-phytase, which initiates phosphate hydrolysis at the 6-position of the phytate molecule, and had an analyzed enzyme activity of 10000 phytase units/g. Phytase II is sourced from *E.coli* and is expressed in *Trichoderma reesei*, produced by company B, Europe, had analyzed enzyme activity of 5000 phytase units/g. and Phytase III is sourced from *Pichia pastoris* yeast, produced by company C, China, as a 3-phytase, which initiates phosphate hydrolysis at the 3-position of the phytate molecule, and had analyzed enzyme activity of 10000 phytase units/g. Enzymatic activity of phytase before and after pellet was determined by the method of Engelen et al. (1994). And the effect of pelleting procedure on the thermo tolerance of different phytase enzyme sources are presented in Table (1).

Experimental birds and diets: Two experiments were done, seven hundred and eighty –day old Arbor Acres broiler chicks were used to study the effect of phytase enzyme supplementation on broiler performance and bone characteristics. First experiment: five hundred and forty chicks were fed diets of 0.3% and 0.27% available P (-40% aP) during starter and grower periods, respectively, in 3×3×2 factorial design, three phytase enzyme sources (Phytase I, 10000U/g; Phytase II, 5000U/g; Phytase III, 10000U/g) in three levels (0, 500 and 750 U/kg diet) with two feed forms either mash or pellet (80°C and pressure load of 40 psi/square inch). Second experiment: one hundred and eighty –one day old- Arbor Acres broiler chicks were fed 0.4% and 0.36% available P(-20% aP) during starter and grower periods, respectively, with 3 levels of phytase enzyme (Phytase I) being (0, 500 and 750 CFU/g) and 2 feed forms (mash and pellet),

and make a comparison with -40% aP with the same levels of Phytase I and feed forms. Thus, the treatments were 2×3×2 factorial design. In addition to two control groups which fed diet of 0.5% and 0.45% available P (strain recommendation of aP) during starter and grower periods, respectively, with two feed forms (mash and pellet) for comparison. Each treatment contained 30 chicks (10 chicks/ replicate). Chicks fed corn-soybean diet according to the experimental design in 2 phases feeding system (starter period from 1 – 15 day and grower period from 16 - 35 day). All diets were formulated to save all strain nutritional requirements except the treatments which differ in available phosphorus (Table 2). Calculated analysis of the diets was done according to feed composition tables for animal and poultry feedstuffs used in Egypt (2001). Feed and water were provided ad lib. At the end of starter period (day 15) and grower period (day 35), chicks were weighed and recorded live body weight, feed intake (FI) and feed conversion ratio (FCR) was calculated according to the following formula:

FCR = feed intake (g)/ body weight gain (g).

Carcass traits and blood samples: Three chicks around the average live body weight of every treatment for every examination, were slaughtered at the end of growing period (35 days of age), then carcass characteristics including carcass, abdominal fat, giblets (liver, gizzard and heart), as percentage of live body weight were recorded. Blood tests were brought into tubes with anticoagulant (heparin) centrifuged at 3000 rpm for 5 minutes and plasma were put away at - 20°C until investigated for phosphorus and calcium by atomic absorption .

Bone qualities: The left and right tibia of each bird were removed as drumsticks with flesh intact. The drumsticks were labeled and immersed in boiling water (100 °C) for 10 min. After cooling to room temperature, the drumsticks were defleshed by hand. They were then air-dried for 24 h at room

temperature. The tibiotarsal length and bone weight were determined. The robusticity index (RI) is determined using the following formula:

Robusticity Index (RI) = bone length / cube root of bone weight Reisenfeld (1972).

To determine toe, foot and tibia ash content were oven-dried at 105°C for 24 h and ashed in a muffle furnace at 600 °C for 6 h according to the procedure of A.O.A.C. (1994). The percentage ash was determined relative to dry weight of the toe, foot and tibia, respectively. And tibia content of Ca and P were determined using commercial kits.

Statistical analysis: data was performed using the general linear model (GLM) procedure of SAS software (SAS Institute, 2001) to detect the effect of main factors and their interaction.

according to the following model:

$Y_{ijkl} = \mu + S_i + L_j + F_k + (SLF)_{ijk} + e_{ijkl}$
where

Y_{ijkl} = observations, μ = overall mean, S_i = phytase source ($i= 1, 2, 3$), L_j = phytase levels ($j= 1, 2, 3$),

F_k = feed form ($k= 1,2$), $(SLF)_{ijk}$ = interaction effect between main factors, e_{ijkl} = experimental error. (Exp.1),

$Y_{ijkl} = \mu + P_i + L_j + F_k + (PLF)_{ijk} + e_{ijkl}$
where

Y_{ijkl} = observations, μ = overall mean, P_i = phosphorus level ($i= 1, 2$), L_j = phytase levels ($j= 1, 2, 3$),

F_k = feed form ($k= 1,2$), $(PLF)_{ijk}$ = interaction effect between main factors, e_{ijkl} = experimental error. (Exp.2)

Three non-orthogonal contrasts were also included to compare stain recommendation of aP vs. either

-40% aP or -20% aP with or without phytase supplementation.

Duncan's Multiple Range test (Duncan's, 1955) was used to separate means when separation was relevant. Statistical significance was accepted at probability level of ($p \leq 0.05$).

RESULTS AND DISCUSSION

First experiment:

Growth performance:Supplementation effect of different phytase sources on the growth performance of broiler chicks are presented in Table (3). Phytase supplementation (Phytase III) improved overall weight gain, increased feed intake at grower and overall periods. While, FCR were not affected comparing to the two other sources. This result agree with Silversides et al., (2004) who concluded that adding phytase (derived from *Escherichia coli* gene but produced in *Pichia pastoris* yeast) to negative control diet (deficient in available P) had positive effects on broiler performance rather than other groups fed diet supplemented with phytase produced ether from *Saccharomyces cerevisiae* or *Pseudomonas fluorescens*. The enhancement due to supplementing Phytase III may be due to that enzyme resist degradation of proteases and autolysis of enzyme so as to increase stability of phytase in bird body. It is not clear why different production systems of the same *E. coli*-derived enzyme produced differences among the enzyme samples. The differences may relate to changes in pelleting stability, in susceptibility to proteolytic enzymes in the intestinal tract, or in specific activity.

Chicks fed 750 FTU/kg phytase supplemental diets recorded the best values of the live body weight at starter and grower periods, it increased FI during grower and overall periods. Also, it improved FCR during all growth periods without significant differences to those fed dietary 500 FTU/kg supplemental diets. This was interpreted as a phytase induced release of phytate-bound P. The capacity of phytase to enhance P accessibility by hydrolyzing phytate-bound P in poultry diet is very much archived (Kornegay et al., 1996; Qian et al., 1997).

As indicated by feed form, chicks fed pelleted diet recorded significantly better values in all growth parameters except FI

during grower period which was not significantly affected. The present results confirms the recently research of Amer et al., (2015) who found that the chicks fed pellets form has highly significant body weight over the chicks fed mash throughout the 8 weeks. Also, confirms the previous results by Jafarnejad et al., (2010) and Zohair et al. (2012) who observed the superiority score of weight for broiler chicks fed pellet diets over those fed mash diet during different stages of fattening periods. These results are not agree with Murakami et al. (2008) who reported that quails fed pellet feed presented higher feed intake as compared to mash fed birds. Also, Preston et al. (2000) and Frikha et al. (2009) reported that laying hens consumed more feed when offered in pellet form compared to mash form. The higher live body weights observed for birds fed the pellet diets may be due to that steam pelleting process might have enhanced the nutritive value, as concluded by Savory (1974).

Chicks fed diets contained -40% aP and supplemented with different levels of phytase significantly lower than the control group (strain recommendation of aP) in final live body weight and overall feed conversion ratio. Our results are in accordance with Shahir et al., (2015) who reported that phosphorus restriction (33% reduction of dietary phosphorus) reduced growth performance of broiler chicks compared with others fed control diet fed 0.5, 0.45 and 0.4% in the starter, grower and finisher periods, respectively.

Carcass characteristics:

Results in Table (4) show that chicks fed diets supplemented with Phytase I and Phytase III recorded the best carcass percentage without significant differences between them. While, those fed diets supplemented with Phytase I and Phytase II recorded significantly higher giblets % than others fed dietary Phytase III. The improvement in carcass % as a result of supplementation of either Phytase I or

Phytase III may be due to that they are tolerated to acidic condition in the gut, where they are effective under the pH ranged between 2.5 – 6.5.

Regarding to dietary phytase levels, chicks fed both levels of 500 or 750 FTU/kg diet recorded higher carcass and abdominal fat percentages without significant difference between them. In this connection, Pillai et al., (2006) found that phytase supplementation significantly increased dressed carcass percentage and abdominal fat of broilers compared with those fed the control diet without phytase supplementation. It may be due to that phytase plays a role in modulating the gut microbiota of chicken (Ptak et al 2015). While, El-Nagmy et al. (2004) found that phytase did not significantly affect broilers carcass yield.

According to form of feed, chicks fed pellet diet recorded significantly higher carcass % .while, it significantly reduced giblets % than those fed mash diet. These results in agreement with Attia et al., (2014) who concluded that percentage of carcass of group fed pellet diet was significantly higher than that of broilers fed the mash diet.

All of groups fed diets contained -40 % aP and enriched with phytase significantly recorded worse values of carcass% and giblets % in contrast with the control group (strain recommendation of aP).

Plasma and tibia phosphorus and calcium: Results in Table (5) show that chicks fed different phytases did not show any significant effect on plasma phosphorus. While, chicks fed dietary Phytase I and Phytase III recorded significantly higher plasma calcium comparing to those fed dietary Phytase II. Also, chicks fed dietary Phytase II supplementation recorded significantly the worst tibia P and Ca content comparing to others fed dietary Phytase I or Phytase III. As mentioned before there is a wide range of pH for Phytase I and Phytase III being 2.5 to 6.5. In the down of digestive system, at higher pH levels, phytate binds to minerals, for example, calcium and trace

elements. As more phosphorus is expelled from phytate, promoting more breakdown of intact IP-6, the less able it is to bind or chelate minerals, starch or proteins either directly or via ionic bridges (Selle & Ravindran, 2007). Decreasing the binding of these compounds through the use of phytase may directly enhance the digestibility not only of phosphorus and divalent cations such as Ca, Zn and Mg, but also indirectly increase energy and nitrogen utilization.

It is perceptible that plasma P and Ca concentrations were gradually increased by increasing dietary enzyme levels. The same trend was achieved in tibia P and Ca content. In this respect, Viveros et al., (2002) and Jalali et al., (2009) reported that Phytase supplementation increased plasma P when added to low dietary available P level, while, phytase reduced the Ca concentration. Also, Lan et al., (2002) concluded the same results for broilers plasma P, but plasma Ca was not.

Chicks fed mash form recorded significantly higher plasma and tibia P and Ca comparing to those fed pellet form. Our results were confirmed by the previous research recorded by Woyengo et al., (2010) who found that pelleting diet did not affect broilers tibia Ca but improved tibia P.

Chicks fed the control diet (strain recommendation of aP) achieved significantly higher values of all blood and tibia phosphorus and calcium comparing to those fed diet containing -40% aP with different levels of phytase supplementation.

Bone measurements: Bone measurements as affected by different phytase sources, levels and feed form are listed in Table (6). Concerning to phytase sources, chicks fed diets supplemented with Phytase I recorded significantly higher toe and tibia ash and chicks fed dietary Phytase III recorded significantly higher foot ash while, those fed diets supplemented with Phytase II recorded significantly the lowest values for toe, foot and tibia ash. Our results are in accordance with Owusu et al.(2007) who found that supplementing Phytase I

(*Schizosaccharomyces pombe*) to broiler diet had an improvement of tibia ash percentage comparing with others fed diets supplemented with uncoated phytase. The response to phytase sources could be attributed to: a) source of microbial phytase and its efficacy in the biological system, b) source and amount of dietary phytate, c) amount of dietary Ca and available P, d) age of birds or period of study and e) method of accessing P equivalency as concluded by Manangi et al. (2009).

Regarding to phytase levels, chicks fed diets supplemented with (750 FTU/kg diet) significantly achieved the best value for toe, foot and tibia ash also, improved RI comparing with other two phytase levels. In this connection, Silversides et al., (2004) reported that increasing the dose of enzyme B (derived from *Pichia pastoris* yeast) in broiler diet resulted in increasing values for toe ash.

Comparing to mash form, chicks fed pellet diet recorded significantly lower toe, foot and tibia ash. On the other hand, improved RI value. These results confirmed the analysis of Phytase In pelleted diets which is reduced by pelleting process as mentioned in Table (1) which indicated that Phytase I is more stable after pelleting than the other two phytase sources II and III. This resistance may be due to new technique for manufacturing this enzyme which allowed being thermo stable up to 95°C / 203°F during pelleting. In contrast with our results, Woyengo et al., (2010) concluded that pelleting diet did not affect broilers tibia ash. Non of all treatments received -40% aP supplemented with different phytase levels (500 or 750 FTU/ kg diet) reached the group fed the control diet (strain recommendation of aP) on toe, tibia or foot ash. Our result are disagree with the finding of Kiiskinen et al., (1994) who found that normal broiler bone mineralization was supported by supplementing phytase at level of 1 000 PU/kg to diets based on wheat, barley, oat and soybean meal with low or zero inorganic phosphorus content .

Second experiment:

Growth performance: Impact of different treatments on growth performance are listed in Table (7). Chicks fed diets contained -20% aP recorded significantly higher live body weight during starter and grower periods than others fed diets contained -40 % aP. the same pattern was observed in feed intake during starter, grower and overall periods. In match with the current findings, Silversides et al., (2004) concluded that chicks fed the phosphorus-adequate (containing 0.4% aP) diet were heavier at 21 d, ate more, and had better feed efficiency than those fed the phosphorus-deficient diet (containing 0.23% aP). Also, Kozlowski et al.,(2010) reported that Ross 308 male chicks recorded a reduction in weight gain by 9.1% for group fed 5.23 and 4.55 total P/kg, 2.54 g and 1.95 g aP /kg during starter and grower periods, respectively comparing to others fed diet containing 6.73 and 6.05 g total P/kg, 4.05 and 3.46 g available P (aP)/kg during the same growth periods.

Feed conversion ratio was improved by 5.6% and 4.1% for grower and overall periods, respectively, for chicks fed diets contained -20% aP comparing to those fed diets contained -40% aP. This result may be due to phosphorus deficiency has been shown to result in reduced appetite (Gills et al., 1948). In this connection, Kozlowski et al.,(2010) found that FC for Ross 308 male chicks was increased by 8.4% in P deficient diet (reduction by 1.5 g/kg) comparing with control group.

Chicks fed dietary phytase at either 500 or 750 FTU/kg diet recorded significantly higher live body weight and better feed conversion ratio during starter, grower and overall periods than chicks fed diets without phytase supplementation. This result agree with Barnard et al., (2015) who found that phytase supplementation significantly increased broilers weight gain and decreased feed conversion ratio for the 7-42d period ($P < 0.05$). In the current study,

the increased digestibility of P in the basal diet (which was deficient in P) by phytase resulted in increased availability of P to the broilers, which could have led to improved appetite of the birds and hence improved feed intake and growth performance. Other studies have also shown improved performance of broilers fed P deficient diets due to phytase supplementation (Onyango et al., 2005; Olukosi et al., 2007).

Comparing to mash diet, live body weight and feed intake values were significantly increased in chicks fed pelleted diets. Also, feed conversion during starter and overall periods significantly improved by 5.4 % and 4.1%, respectively for those fed pellet diets. Our results agreed with Zohair et al. (2012) who reported that FI, BWG, FCR and performance index were significantly improved with feeding pellet diet compared to feeding mash diet for broiler chicks. The improvements of pellet in performance have been attributed to decrease feed wastage, reduced selective feeding, destruction of pathogenic organisms, improved palatability (Salari et al. 2006), increased nutrient digestibility (Behnke, 1998). Moreover, these observed results explained by recent broiler behavioral studies, since they reported that broilers respond to pelleted feed by spending less time to eat the same or more feed. This decreased the time spent for resting, which decreases bird energy available for gain (Wiernusz, 2012). Contrasting with the control group, chicks fed diets contained -20% P + 500 FTU/kg diet significantly reached the control group while, those fed dietary -20 % aP + 750 FTU/kg diet significantly exceed the control group. Increased utilization of P from phytate can therefore reduce supplementation of diets with inorganic P sources while maintaining normal growth of the bird. Numerous researchers have observed an improvement, due to dietary phytase supplementation, in BW gain and feed intake during the first 21 d of age (Sebastian et al., 1996; Cabahug et al.,

1999), whereas others reported no effect (Perney et al., 1993; Boling-Frankenbach et al., 2001). These differentiating results might be due to various factors including phytase source, ingredients (type, source, phytate content), and dietary characteristics (processing, Vitamin D3 level, Ca:P ratio) (Ravindran et al., 1995a).

Carcass characteristics: Results in Table (8) show that, Regarding to phosphorus levels, chicks fed diets contained -20% P recorded significantly higher carcass percentage while, it recorded significantly lower giblets % than those fed diets contained -40% aP.

Chicks fed diets supplemented with phytase at either 500 or 750 FTU /kg diet recorded significantly higher carcass% than others fed diets without phytase supplementation. These results are similar to those reported by Salem et al. (2003) who concluded that the improvement in carcass yield due to phytase supplementation is a reflection of the increase in nutrient availability for tissue growth.

In respect of diet form, chicks fed pellet diets increased carcass percentage by 1.8% while, it decreased giblets percentage by 10.7% comparing to chicks fed mash diets. All main factors did not show any significant effect on abdominal fat. In this respect, Adeyemi et al. (2008) reported that higher percentage of dressing, breast meat, drumstick and thigh, which are the most expensive commercial cuts of the chicken, were obtained in birds fed pelleted diets. On the other hand, Hassan & El-Sheikh (2010) showed that both carcass and giblets percentages were not affected by feed form. These results confirm the previous results in Table (7) which growth performance of chicks improved by feeding diets containing -20% aP + 500 FTU/kg diet in pelleted form. Chicks fed -20% aP without phytase supplementation recorded lower carcass and abdominal fat percentage than the control. While, those fed diets contained -20 % aP supplemented with either 500 or 750 FTU/kg diet recorded insignificantly

different with the control group in the same items. The reduction in P concentration significantly affected pH in the crop and caeca, reduced butyrate- but increased lactate-producing bacteria (Ptak et al., 2015) and addition of phytase to the deficient P diet increased ileal total bacterial counts.

Plasma and tibia phosphorus and calcium: Effect of different treatments on plasma and tibia phosphorus and calcium are presented in Table (9). Chicks fed dietary -20% aP recorded significantly higher plasma P and Ca, the improvement was 34.8% and 27.7% respectively, comparing with those fed diets contained -40% aP. The same trend was observed in tibia P and Ca which confirmed the results recorded in plasma minerals. Our results agree with Kozłowski et al.,(2010) who concluded that phosphorus reduction in the diets negatively influenced the process of bone mineralization. While, disagree with Silversides et al., (2004) who reported that there were no significant effect between chicks fed adequate phosphorus diet (0.4% aP) and deficient diet (0.23% aP) on sera calcium or phosphorus.

By increasing dietary Phytase I level, the retention of P and Ca in plasma and tibia were significantly increased. Tibia ash is considered to be the most sensitive criterion for assessing response to P availability in poultry (Onyango et al., 2005). Phytase supplementation to P-deficient diets has been shown repeatedly to improve tibia ash content; a response often attributed to improved P digestibility due to phytase supplementation (Olukosi et al., 2007).

Regarding to feed form, chicks fed pellet diet recorded significantly lower P in plasma and tibia comparing to those fed mash diet. On the other hand, Ca concentration in plasma did not affected significantly by feed form. While, Ca concentration in tibia significantly higher in chicks fed mash diet than others fed pellet diet. Our results are in accordance with Kirkpinar and Basmacioğlu (2006) who reported that, tibia P and Ca did not influenced significantly by feeding

broiler chicks with pellet diet. Also, noted that no impacts of pelleting temperatures on Ca content in the serum. However, P content in the serum was increased by feeding the diet pelleted at 65°C as compared to the control (mash diet) and other treatments (pelleting temperatures on 75 and 85 °C) . This negative effect of pellet on plasma and bone phosphorus can be explained by Takemasa and Hijikuro (1983) who showed that steam pelleting of corn-soybean diets had no effect on the availability of phytate P to chickens.

Chicks fed -20% aP + 750 FTU/kg diet reached the control group (strain recommendation of aP) for P and Ca content in plasma and tibia. In this connection, Silversides et al., (2004) have reported improved Ca digestibility due to phytase supplementation. Phytate forms insoluble complexes with Ca at neutral pH found in the small intestine (Maenz et al., 1999), and hence by hydrolyzing phytate, phytase is expected to result in increased Ca digestibility. Whereas, Woyengo et al., (2008) have reported lack of effect of phytase on Ca digestibility. It is thus not clear why phytase has improved Ca digestibility in some studies, but not in others.

Bone estimations:

Results in Table (10) demonstrate that, chicks fed diets containing -20% aP recorded significantly higher toe, foot and tibia ash and enhanced RI value than those fed diets contained -40% aP. Our results are disagree with the previous research of Silversides et al.,(2004) who noted that toe ash didn't affected significantly by feeding broiler chicks with adequate or deficient phosphorus diet.

Toe, foot and tibia ash were significantly increased by increasing dietary Phytase I level. While, RI did not affected significantly. In this connection, Walk et al.

(2011) reported that phytase supplementation increased tibia ash by approximately 3% in broiler chicks. But in contrast to the results obtained by Pintar et al. (2004) who found that supplemental phytase did not influence broilers tibia ash. This different observation can be explained by Underwood's finding (1981) who found that the Ca to non phytate phosphorus ratio beyond 2: 1 reduces bioavailability of Ca and P due to the formation of insoluble calcium-phosphate complex in the chicken gut.

Chicks fed mash diets recorded significantly higher toe and tibia ash than those fed pellet diets. On the other hand, neither foot ash nor RI value affected significantly by feed form. In contrast to our results, Kirkpinar and Basmacioğlu (2006) found that no significant effect of pelleting diet on broilers tibia ash.

Feeding chicks with diets contained -20% aP +500 FTU/kg diet Phytase I recorded an improvement in toe, foot and tibia ash without significant effect to control group. These results are in accordance with Manangi et al. (2009) who found that phytase supplementation with 750 and 1,000 units/kg to low phosphorus diet increased broiler tibia ash % compared to the tibia ash % of broilers fed the control diet.

Recommendation: addition of coated phytase (Phytase I) derived from *Schizosaccharomyces pombe* at level of 500 FTU/kg diet to diet contained -20% aP enhanced growth performance, bone mineralization of broiler chicks fed corn-soybean-based diets in mash or pellet form, indicating that the bio efficacy of the coated phytase used in the current study was not affected by the pelleting process. Consequently, the coated phytase used in the current study can be supplemented to diets that are to be pelleted.

Table (1) :Different sources of phytase activity before and after pelleting

Phytase sources	Mash diet	Pellet diet
Phytase I (10000 U/g)	752 U/kg diet	417 U/kg diet
Phytase II (5000 U/g)	748 U/kg diet	331 U/kg diet
Phytase III (10000 U/g)	750 U/kg diet	300 U/kg diet

Broilers- Pellet diet- Mash diet- Thermo-stable phytase- Growth performance.

Table (2): Feed ingredients and calculated analysis of the experimental diets.

Ingredients	Starter period (1-15 day of age)			Grower period (16-35 day of age)		
	Available Phosphorus level (%)			Available Phosphorus level (%)		
	0.5(strain Recommendation)	0.4 (-20%)	0.3 (-40%)	0.45 (strain Recommendation)	0.36 (-20%)	0.27 (-40%)
Yellow corn	52.98	53.37	53.30	60.76	60.75	60.60
Soybean meal	31.9	32.52	33.23	22.65	23.10	23.80
Corn gluten meal	7.64	6.91	6.44	9.72	9.25	8.78
Soybean oil	2.50	2.50	2.50	2.50	2.70	2.80
Di calcium phosphate	1.93	1.37	0.81	1.72	1.21	0.71
Lime stone	1.39	1.67	2.08	1.10	1.45	1.80
NaCl	0.45	0.45	0.45	0.45	0.45	0.45
Vit. &min. mix	0.3	0.30	0.30	0.30	0.30	0.30
D.L. Methionine	0.29	0.30	0.30	0.19	0.20	0.20
L. Lysine HCl	0.51	0.50	0.48	0.52	0.50	0.48
L. Therionine	0.11	0.11	0.11	0.09	0.09	0.08
Total	100	100	100	100	100	100
Calculated analysis**						
Crude protein %	23.1	23.1	23.1	21.1	21.1	21.1
ME kcal/kg	3025	3025	3025	3150	3150	3150
Crude fiber %	3.59	3.64	3.69	3.15	3.16	3.21
Crude fat %	5.02	5.03	5.02	5.28	5.27	5.27
Calcium %	1.05	1.05	1.05	0.90	0.90	0.90
Available phosphorus %	0.50	0.40	0.30	0.45	0.36	0.27
Lysine %	1.43	1.43	1.43	1.24	1.24	1.24
Methionine %	0.73	0.73	0.73	0.62	0.62	0.62
Meth. + Cys.	1.07	1.07	1.07	0.95	0.95	0.95
Therionine %	0.94	0.94	0.94	0.83	0.83	0.83
Sodium %	0.19	0.19	0.19	0.19	0.19	0.19

Each 3 kg contain: Vit A12 000 000 IU, Vit D₃ 2 000 000IU, Vit E 10g, Vit K₃ 2g, Vit B₁ 1g, Vit B₂ 5g, Vit B₆ 1.5g, Vit B₁₂ 10mg, Nicotinic acid 30g, Pantothenic acid 10g, Folic acid 1g, Biotin 50mg, Choline chloride 250g, Iron 30g, Copper 10g, Zinc 50g, Manganese 60g, Iodine 1g, Selenium 0.1g, Cobalt 0.1g and carrier (CaCO₃) up to 3 kg.

**According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001).

Table (3): Effect of experimental treatments (Exp.1) on body weight, feed intake and feed conversion

Item Age	Body weight/g			Feed intake/g			Feed conversion		
	1 day	2 week	5 week	2 week	5 week	0-5 week	2 week	5 week	0-5 week
SR of aP (Control)	52	381	1832	468	2390	2859	1.42	1.65	1.61
Main effects:									
Phytase Source:									
Phytase I	51	361	1430 ^b	449	1885 ^b	2334 ^c	1.45	1.76	1.69
Phytase II	52	366	1461 ^b	463	1963 ^b	2426 ^b	1.47	1.79	1.72
Phytase III	51	368	1549 ^a	458	2053 ^a	2512 ^a	1.44	1.74	1.68
SEM	-	5.74	20.52	6.93	24.08	26.90	0.012	0.016	0.014
P _≤	NS	NS	<.0001	NS	0.0006	0.0004	NS	NS	NS
Phytase Level:									
0 (FTU/kg diet)	51	349 ^b	1348 ^c	448	1831 ^b	2280 ^b	1.50 ^a	1.83 ^a	1.76 ^a
500 (FTU/kg diet)	52	370 ^a	1507 ^b	458	1999 ^a	2457 ^a	1.44 ^b	1.76 ^b	1.69 ^b
750 (FTU/kg diet)	52	378 ^a	1585 ^a	464	2071 ^a	2535 ^a	1.42 ^b	1.72 ^b	1.65 ^b
SEM	-	5.74	20.52	6.93	24.08	26.90	0.012	0.016	0.014
P _≤	NS	<.0001	<.0001	NS	<.0001	<.0001	0.013	0.022	0.014
Feed form:									
Mash form	51	330 ^b	1404 ^b	417 ^b	1936	2354 ^b	1.49 ^a	1.80 ^a	1.74 ^a
Pelleted form	52	402 ^a	1556 ^a	496 ^a	1998	2494 ^a	1.42 ^b	1.73 ^b	1.66 ^b
SEM	-	5.74	20.52	6.93	24.08	26.90	0.012	0.016	0.014
P _≤	NS	<.0001	<.0001	<.0001	NS	0.0001	0.003	0.032	0.006
Effect of the interaction between:									
Phy. S* Phy. L	NS	NS	<.0001	NS	0.027	0.0141	NS	NS	NS
F* Phy. S	NS	NS	0.008	NS	NS	NS	NS	NS	NS
F* Phy. L	NS	NS	NS	NS	NS	NS	NS	NS	NS
F*Phy.S* Phy L	NS	0.015	0.058	NS	NS	NS	NS	NS	NS
Non orthogonal contrast									
SR vs. 0	NS	NS	0.0001	NS	0.0002	0.0003	0.05	0.02	0.03
SR vs. 500	NS	NS	0.0001	NS	0.0001	0.0001	NS	0.02	0.05
SR vs. 750	NS	NS	0.001	NS	0.001	0.004	NS	NS	NS

a,b,...= Means in the same column with different superscripts, differ significantly (p<0.05); NS = Not Significant (p>0.05); SEM=Standard Error of Means.

Phy.S= phytase source, Phy.L= phytase level, F= feed form, SR= strain recommendation of available Phosphorus.

Broilers- Pellet diet- Mash diet- Thermo-stable phytase- Growth performance.

Table (4): Effect of experimental treatments (Exp.1) on carcass characteristics (% live body weight).

Item	Carcass (%)	Giblets (%)	Abdominal fat (%)
SR of aP (Control)	71.82	4.59	1.69
Main effects:			
Phytase source			
Phytase I	69.42 ^a	5.20 ^a	1.50
Phytase II	68.08 ^b	5.36 ^a	1.55
Phytase III	69.94 ^a	4.87 ^b	1.58
SEM	0.267	0.076	0.055
P≤	0.003	0.014	N.S
Phytase level:			
0 (FTU/kg diet)	67.61 ^b	5.19	1.38 ^b
500 (FTU/kg diet)	69.45 ^a	5.15	1.73 ^a
750 (FTU/kg diet)	69.96 ^a	5.11	1.49 ^{ab}
SEM	0.267	0.076	0.055
P≤	0.0002	NS	0.0510
Feed form:			
Mash form	68.70 ^b	5.34 ^a	1.51
Pelleted form	69.61 ^a	4.93 ^b	1.57
SEM	0.267	0.076	0.055
P≤	0.042	0.003	NS
Effect of the interaction between:			
Phy. S* Phy. L	NS	0.049	NS
F* Phy. S	NS	NS	NS
F* Phy. L	NS	NS	NS
F*Phy.S* Phy. L	NS	NS	NS
Non orthogonal contrast			
SR vs. 0	0.0004	0.02	NS
SR vs. 500	0.005	0.04	NS
SR vs. 750	0.05	0.04	NS

a,b,...= Means in the same column with different superscripts, differ significantly (p<0.05);

N.S = Not Significant (p>0.05); SEM=Standard Error of Means.

Phy.S= phytase source, Phy.L= phytase level, F= feed form, SR= strain recommendation of available phosphorus.

Table (5): Effect of experimental treatments (Exp.1) on plasma and tibia phosphorus and calcium

Item	Plasma		Tibia	
	Phosphorus (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/g)	Calcium (mg/g)
SR of aP (Control)	2.82	8.40	37.64	41.47
Main effects:				
Phytase source:				
Phytase I	1.582	6.026 ^a	33.13 ^a	38.88 ^b
Phytase II	1.508	5.662 ^b	32.16 ^c	38.04 ^c
Phytase III	1.677	6.168 ^a	32.82 ^b	39.08 ^a
SEM	0.07	0.11	0.07	0.06
P _≤	NS	0.0091	0.0001	0.0001
Phytase level				
0 (FTU/kg diet)	1.309 ^c	5.413 ^c	29.48 ^c	37.90 ^c
500 (FTU/kg diet)	1.541 ^b	5.977 ^b	33.52 ^b	38.51 ^b
750 (FTU/kg diet)	1.918 ^a	6.468 ^a	35.10 ^a	39.60 ^a
SEM	0.07	0.11	0.07	0.06
P _≤	0.0001	0.0001	0.0001	0.0001
Feed form				
Mash form	1.793 ^a	6.172 ^a	32.94 ^a	39.11 ^a
Pelleted form	1.385 ^b	5.733 ^b	32.46 ^b	38.23 ^b
SEM	0.07	0.11	0.07	0.06
P _≤	0.0001	0.0018	0.0001	0.0001
Effect of the interaction between:				
Phy. S* Phy. L	NS	0.0096	0.0001	0.0001
F* Phy. S	NS	NS	NS	0.0001
F* Phy. L	0.0018	0.0524	0.0001	0.0001
F*Phy.S* Phy. L	NS	NS	NS	0.0001
Non orthogonal contrast				
SR vs. 0	0.0001	0.0001	0.0001	0.0001
SR vs. 500	0.0001	0.0001	0.0001	0.0002
SR vs. 750	0.0001	0.0001	0.0001	0.0029

a,b,...= Means in the same column with different superscripts, differ significantly (p<0.05);

NS = Not Significant (p>0.05); SEM=Standard Error of Means.

Phy.S= phytase source, Phy.L= phytase level, F= feed form, SR= strain recommendation of available phosphorus.

Broilers- Pellet diet- Mash diet- Thermo-stable phytase- Growth performance.

Table (6): Effect of experimental treatments (Exp.1) on bone measurements

Item	Toe ash (%)	Foot ash (%)	Tibia ash (%)	RI
SR of aP (Control)	11.93	11.81	28.67	4.99
Main effects:				
Phytase source:				
Phytase I	9.61 ^a	8.88 ^b	25.04 ^a	5.11
Phytase II	8.92 ^b	8.49 ^c	23.46 ^c	5.09
Phytase III	9.02 ^b	9.59 ^a	24.31 ^b	5.05
SEM	0.06	0.09	0.17	0.05
P _≤	0.0001	0.0001	0.0001	NS
Phytase level:				
0 (FTU/kg diet)	8.24 ^c	7.42 ^b	23.62 ^b	5.23 ^a
500 (FTU/kg diet)	9.33 ^b	9.80 ^a	23.85 ^b	5.08 ^b
750 (FTU/kg diet)	10.00 ^a	9.75 ^a	25.35 ^a	4.94 ^c
SEM	0.06	0.09	0.17	0.05
P _≤	0.0001	0.0001	0.0001	0.0004
Feed form:				
Mash form	9.44 ^a	9.26 ^a	25.00 ^a	5.14 ^a
Pelleted form	8.92 ^b	8.72 ^b	23.55 ^b	5.03 ^b
SEM	0.06	0.09	0.17	0.05
P _≤	0.0001	0.0001	0.0001	0.051
Effect of the interaction between:				
Phy. S* Phy. L	0.0001	0.0001	0.0001	NS
F* Phy. S	NS	0.002	0.0001	NS
F* Phy. L	0.0001	0.0001	0.0001	NS
F*Phy.S* Phy. L	NS	NS	0.0006	NS
Non orthogonal contrast				
SR vs. 0	0.0001	0.0001	0.0001	NS
SR vs. 500	0.0001	0.003	0.003	0.0001
SR vs. 750	0.0007	0.0003	0.0007	NS

a,b,...= Means in the same column with different superscripts, differ significantly (p<0.05);
N.S = Not Significant (p>0.05); SEM=Standard Error of Means.

Phy.S= phytase source, Phy.L= phytase level, F= feed form, SR= strain recommendation of available phosphorus.

Table (7): Effect of experimental treatments (Exp. 2) on body weight, feed intake and feed conversion

Item	Body weight			Feed intake			Feed conversion		
	1 day	2 week	5 week	2 week	5 week	0-5 week	2 week	5 week	0-5 week
SR of aP (Control)	52	381	1832	468	2390	2859	1.42	1.65	1.61
-20% aP	52	357	1723	451	2353	2804	1.48	1.72	1.68
-20% aP + 500	52	390	1812	484	2353	2837	1.43	1.65	1.61
-20% aP + 750	52	404	1850	497	2354	2842	1.41	1.63	1.58
Main effects									
Available Phosphor Level:									
-20%	52	383 ^a	1795 ^a	477 ^a	2350 ^a	2827 ^a	1.44	1.66 ^b	1.62 ^b
-40%	51	361 ^b	1430 ^b	449 ^b	1885 ^b	2334 ^b	1.45	1.76 ^a	1.69 ^a
SEM	-	7.23	38.22	8.35	46.10	50.41	0.013	0.019	0.016
P<	NS	<.0001	<.0001	0.002	<.0001	<.0001	NS	0.007	0.019
Phytase level:									
0 (FTU/kg diet)	51	353 ^b	1536 ^b	449	2092	2542	1.49 ^a	1.77 ^a	1.71 ^a
500 (FTU/kg diet)	52	378 ^a	1625 ^a	468	2120	2588	1.44 ^{ab}	1.70 ^{ab}	1.65 ^{ab}
750 (FTU/kg diet)	52	385 ^a	1676 ^a	472	2141	2613	1.42 ^b	1.66 ^b	1.61 ^b
SEM	-	±7.23	±38.22	±8.35	±46.10	±50.41	±0.013	±0.019	±0.016
P≤	NS	<.0001	<.0001	NS	NS	NS	0.045	0.041	0.023
Feed form:									
Mash form	51	337 ^b	1506 ^b	426 ^b	2027 ^b	2453 ^b	1.49 ^a	1.73	1.69 ^a
Pelleted form	52	406 ^a	1719 ^a	500 ^a	2207 ^a	2708 ^a	1.41 ^b	1.68	1.62 ^b
SEM	-	7.23	38.22	8.35	46.10	50.41	0.013	0.019	0.016
P≤	NS	<.0001	<.0001	<.0001	0.0001	<.0001	0.005	NS	0.05
Effect of the interaction between:									
Pho.L*Phy.L	NS	0.042	NS	NS	NS	NS	NS	NS	NS
Pho.L*F	NS	NS	NS	NS	NS	NS	NS	NS	NS
phy.L*F	NS	0.013	NS	NS	NS	NS	NS	NS	NS
Pho.L*	NS	0.001	NS	0.013	NS	NS	NS	NS	NS
phy.L*F									
Non orthogonal contrast									
SR vs. -20%	NS	NS	NS	NS	NS	NS	NS	NS	NS
SR vs. -20% + 500	NS	NS	NS	NS	NS	NS	NS	NS	NS
SR vs. -20% + 750	NS	NS	NS	NS	NS	NS	NS	NS	NS

a,b,...= Means in the same column with different superscripts, differ significantly ($p < 0.05$); N.S = Not Significant ($p > 0.05$); SEM=Standard Error of Means.

Pho.L = Phosphorus level, Phy. L = Phytase level, F = Feed form, SR= strain recommendation of available phosphorus.

Broilers- Pellet diet- Mash diet- Thermo-stable phytase- Growth performance.

Table (8): Effect of experimental treatments (Exp.2) on carcass characteristics (% live body weight)

Item	Carcass (%)	Giblets (%)	Abdominal fat (%)
SR of aP (Control)	71.82	4.59	1.69
-20% aP	70.25	4.64	1.64
-20% aP + 500	72.17	4.87	1.13
-20% aP + 750	72.56	4.63	1.84
Main effects:			
Available Phosphor Level:			
-20%	71.67 ^a	4.71 ^b	1.51
-40%	69.42 ^b	5.20 ^a	1.50
SEM	0.377	0.087	0.083
P _≤	0.0005	0.001	N.S
Phytase Level:			
0 (FTU/kg diet)	69.03 ^b	4.89	1.51
500 (FTU/kg diet)	71.01 ^a	5.12	1.37
750 (FTU/kg diet)	71.50 ^a	4.82	1.65
SEM	0.377	0.087	0.083
P _≤	0.005	N.S	N.S
Feed Form:			
Mash form	69.89 ^b	5.25 ^a	1.41
Pelleted form	71.16 ^a	4.69 ^b	1.58
SEM	0.377	0.087	0.083
P _≤	0.038	0.0003	N.S
Effect of the interaction between:			
Pho.L*Phy.L	NS	NS	NS
Pho.L*F	NS	NS	NS
phy.L*F	NS	NS	NS
Pho.L* phy.L*F	NS	NS	NS
Non orthogonal contrast			
SR vs. -20%	NS	NS	NS
SR vs. -20% + 500	NS	NS	0.01
SR vs. -20% + 750	NS	NS	NS

a,b,...= Means in the same column with different superscripts, differ significantly (p<0.05); NS = Not Significant (p>0.05); SEM=Standard Error of Means.

Pho.L = Phosphorus level, Phy. L = Phytase level, F = Feed form, SR= strain recommendation of available phosphorus.

Table (9): Effect of different treatments (Exp.2) on plasma and tibia phosphorus and calcium

Item	Plasma		Tibia	
	Phosphorus (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/g)	Calcium (mg/g)
SR of aP (Control)	2.82	8.40	37.64	41.47
-20% aP	1.83	7.24	36.03	38.69
-20% aP + 500	1.97	7.69	36.28	39.00
-20% aP + 750	2.52	8.17	37.95	40.0
Main effects:				
Available Phosphorus levels				
-20 %	2.13 ^a	7.70 ^a	36.75 ^a	39.24 ^a
-40 %	1.58 ^b	6.03 ^b	33.13 ^b	38.89 ^b
SEM	0.10	0.11	0.10	0.07
P _≤	0.0013	0.0001	0.0001	0.002
Phytase levels				
0 (FTU/kg diet)	1.55 ^b	6.33 ^c	32.75 ^c	38.30 ^c
500 (FTU/kg diet)	1.65 ^b	6.87 ^b	35.10 ^b	38.94 ^b
750 (FTU/kg diet)	2.31 ^a	7.39 ^a	36.97 ^a	39.96 ^a
SEM	0.10	0.11	0.10	0.07
P _≤	0.0004	0.0001	0.0001	0.0001
Feed form				
Mash form	2.09 ^a	6.97	35.12 ^a	39.30 ^a
Pelleted form	1.62 ^b	6.76	34.76 ^b	38.30 ^b
SEM	0.10	0.11	0.10	0.07
P _≤	0.0045	NS	0.023	0.0001
Effect of the interaction between:				
Pho.L*Phy.L	NS	NS	0.0001	0.018
Pho.L*F	NS	NS	NS	NS
phy.L*F	0.0167	NS	0.0003	0.0001
Pho.L* phy.L*F	NS	NS	NS	0.0064
Non orthogonal contrast				
SR vs. -20%	0.002	0.0006	0.0006	0.0001
SR vs. -20% +500	0.0199	0.0305	0.0037	0.0001
SR vs. -20% +750	NS	NS	NS	0.0033

a,b,...= Means in the same column with different superscripts, differ significantly (p<0.05);

N.S = Not Significant (p>0.05); SEM=Standard Error of Means.

Pho.L = Phosphorus level, Phy. L = Phytase level, F = Feed form, SR= strain recommendation of available phosphorus.

Broilers- Pellet diet- Mash diet- Thermo-stable phytase- Growth performance.

Table (10) :Effect of experimental treatments (Exp.2) on bone measurements

Item	Toe ash (%)	Foot ash (%)	Tibia ash (%)	RI
SR of aP (control)	11.93	11.81	28.67	4.99
-20% aP	10.77	10.23	26.34	4.77
-20% aP + 500	11.11	11.01	30.61	5.09
-20% aP + 750	10.45	11.02	32.54	5.14
Main effects				
Available Phosphorus levels				
-20%	10.78 ^a	10.75 ^a	29.83 ^a	5.00
-40%	9.61 ^b	8.88 ^b	25.04 ^b	5.11 ^a
SEM	0.07	0.10	0.23	0.04
P _≤	0.0001	0.0001	0.0001	0.05
Phytase levels:				
0 (FTU/kg diet)	9.51 ^b	8.82 ^c	24.98 ^c	5.00
500 (FTU/kg diet)	10.54 ^a	10.11 ^b	28.21 ^b	5.08
750 (FTU/kg diet)	10.62 ^a	10.52 ^a	29.12 ^a	5.07
SEM	0.07	0.10	0.23	0.04
P _≤	0.0001	0.0001	0.0001	NS
Feed form:				
Mash form	10.42 ^a	9.93	28.89 ^a	5.10
Pelleted form	10.02 ^b	9.71	25.98 ^b	5.01
SEM	0.07	0.10	0.23	0.04
P _≤	0.0001	NS	0.0001	NS
Effect of the interaction between:				
Pho.L*Phy.L	0.0001	0.0001	0.0002	0.0007
Pho.L*F	NS	NS	NS	NS
phy.L*F	0.0001	NS	0.0001	NS
Pho.L* phy.L*F	0.0228	NS	NS	NS
Non orthogonal contrast				
SR vs. -20%	0.0052	0.0049	0.0024	0.019
SR vs. -20% +500	NS	NS	NS	NS
SR vs. -20% +750	0.0028	NS	0.002	NS

a,b,...= Means in the same column with different superscripts, differ significantly (p<0.05);

NS = Not Significant (p>0.05); SEM=Standard Error of Means. Pho.L = Phosphorus level, Phy. L = Phytase level, F = Feed form, SR= strain recommendation of available phosphorus.

REFERENCES

- Adeyemi, O.A., Eruvbetine D., Oguntona T., Dipeolu M, Agunbiade J.A. 2008.** Feeding broiler chicken with diets containing whole cassava root meal fermented with rumen filtrate. *Arch Zootec* 57, 247-258.
- Amer F.M., Soliman F.N., Bahie El-Deen M. and Azza El-Sebai. 2015.** Effect of diet form and litter types on the productive traits of broiler (Sasso). *Egypt. Poult. Sci.* 35(III): 719-734.
- Association of Official Analytical Chemist (AOAC) 1994.** Official Method of Analysis (12 edition) Washington D.C USA.
- Attia, Y.A.; El-Tahawy, W. S.; Abd El-Hamid, A. E.; Nizza, A.; Al-Harathi, M.A.; El-Kelway, M. I. and Bovera, F. 2014.** Effect of feed form, pellet diameter and enzymes supplementation on carcass characteristics, meat quality, blood plasma constituents and stress indicators of broilers. *Archiv Tierzucht* 57 : 30, 1-14.
- Barnard, L.; Khattak, F. and Romero, L. 2015.** A value-based approach to determine the optimal dose of an e. coli phytase in terms of p replacement and improvements in broiler performance. 20th European Symposium on Poultry Nutrition | 24–27 August 2015 | Prague, Czech Republic.
- Bedford M.R. 2000.** Exogenous enzymes in monogastric nutrition- their current value and future benefits. *Animal Feed Science Technology*.8:1-13.
- Behnke, K.C. 1998.** Why pellet? In Proc. Kansas state University/American Feed Industry Assoc. Pellet conference, Manhattan, KS.
- Boling-Frankenbach, S. D., Peter, C. M., Douglas, M. W., Snow, J. L., Parsons, C. M. and Baker, D. H. 2001.** Efficacy of phytase for increasing protein efficiency ratio values of feed ingredients. *Poult. Sci.* 80:1578–1584.
- Cabahug, S., Ravindran, V., Selle, P. H. and Bryden, W. L. 1999.** Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and nonphytate phosphorus contents. I. Effects on bird performance and toe ash. *Br. Poult. Sci.* 40:660–666.
- Dos Santos, T.T., Srinongkote, S., Bedford, M.R. and Walk, C.L. 2013.** Effect of high phytase inclusion rates on performance of broilers fed diets not severely limited in available phosphorus. *Asian-Aust. J. Anim.Sci.* 26(2): 227-232.
- Duncan, D.B. 1955.** Multiple range and multiple F tests. *Biometrics*, 11: 1-42.
- El-Nagmy, K., Abd El-Samee, M. and Ibrahim, M. 2004.** Effect of dietary plant protein and microbial phytase levels on performance of broiler chicks *Egypt. Poult. Sci.*, 24: 101-121.
- Engelen, A. J., van der Heeft, F. C., Randsdorp, P. H. G. and Smit, E. L. C. 1994.** Simple and Rapid Determination of Phytase Activity. *J. AOAC Int.* 77:760–764.
- Feed Composition Tables For Animal and Poultry Feedstuffs Used In Egypt, 2001.** Technical bulletin No.1, central lab for Feed and food, ministry of agriculture, Egypt.
- Frikha, M., Safaa, H. M., Serrano, M.P., Arbe, X., Mateos, G.G. 2009.** Influence of main cereal of the diet and feed form of growth performance and digestive traits of brown-egg laying pullets. *Poult. Sci.* 88, 994-1002.
- Gillis M.B., Norris L.C. and Heuser G.F.1948.** The utilization y the chick of phosphorus from di erent sources. *Journal of Nutrition*, 35: 195-207.
- Hassan M.M, El-Sheikh S.E.M. 2010.** Effect of physical form of diets on the performance of ducks. *Egypt Poult Sci J* 30, 989-1002.
- Jafarnejad, S.; Farkhoy, M. ; Sadegh, M. and Bahonar, A.R. 2010.** Effect of crumble-pellet and mash diets with different levels of dietary protein and

- energy on the performance of broilers at the end of the third week. *Vet. Med. Inter. Article* 328123. <http://www.hindawi.com/journals/vmi/2010/328123>
- Jalali, S., Jafarian, M. and Tavakoli, S. 2009.** Effects of supplemental dietary phytase on performance and some blood biochemical parameter of broiler chicks 5th Int. Poult. Conf., Egypt, pp.675-679.
- Kiiskinen T., Piironen J., Hakonen T. 1994.** Effects of supplemental microbial phytase on performance of broiler chickens. *Agric. Sci. Finland*, 3, 457–465.
- Kirkpinar, F. and Basmacioğlu, H. 2006.** Effects of pelleting temperature of phytase supplemented broiler feed on tibia mineralization, calcium and phosphorus content of serum and performance. *Czech J. Anim. Sci.*, 51(2): 78–84.
- Kornegay, E. T., Yi, Z., Ravindran, V. and Denbow, D. M. 1996.** Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. *Poult. Sci.* 75:240–249.
- Kozłowski K., Jankowski J. and Jeroch H. 2010.** Efficacy of different levels of *Escherichia coli* phytase in broiler diets with a reduced P content. *Pol. J. Vet. Sci.* 13(3):431-6.
- Lan, G., Abdullah, N., Jalaludin, S. and Ho, Y. 2002.** Efficacy of supplementation of a phytase-producing bacterial culture on the performance and nutrient use of broiler chickens fed corn-soybean meal diet. *Poult. Sci.*, 81:1522-1532.
- Lenis N.P. and Jongbloed A.W. 1999.** New technologies in low pollution swine diets: Diet manipulation and use of synthetic amino acids, phytase and phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emission - review. *Asian-Australian Journal of Animal Science*, 12:305-327.
- Maenz D.D., Engele-Schaan C.M., Newkirk R.W. and Classen H.L. 1999.** The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a lurry of canola meal. *Animal Feed Science and Technology*, 81:177-192.
- Manangi ,M.K., Sands, J.S. and Coon, C.N. 2009.** Effect of Adding Phytase to Broiler Diets Containing Low and High Phytate Phosphorus: 1. Performance, Phytate P Hydrolysis, Tibia Ash, Litter Phosphorus and Ca and P Digestion and Retention. *International Journal of Poultry Science* 8 (10): 919-928.
- Murakami, A., Souza, L., Sakamoto, M. and Fernandes, J. 2008.** Using processed feeds for laying quails (*Coturnix coturnix japonica*). *Braz. J. Poult. Sci.*, 10 (4):205 – 208.
- Olukosi A.O., Cowieson A.J. and Adeola O. 2007.** Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. *Poultry Science*, 86: 77-86.
- Onyango E.M., Bedford M.R. and Adeola O. 2005.** Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. *Poultry Science*, 84:248-255.
- Owusu, A.A., Gilbert, C., Simmins, P.H., and Nyachoti, C.M. 2007.** Effect of a new coating on the bioefficiency of phytase product in broilers fed corn-soybean meal based diets. 16th European Symposium on Poultry Nutrition.
- Perney, K. M., Cantor, A. H., Straw, M. L. and Herkelman, K. L. 1993).** The effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. *Poult. Sci.* 72:2106–2114.

- Pillai, P., Connor-Dennie, T., Owens, C. and Emmert, J. 2006.** Efficacy of an *Escherichia coli* phytase in broilers fed an adequate or reduced phosphorus diets and its effect on carcass characteristics. *Poult. Sci.*, 85:1737-1745.
- Pintar, J., Homen, B., Gazic, K., Grbesa, D., Sikiric, M. and Cerny, T. 2004.** Effects of supplemental phytase on performance and tibia ash of broilers fed different cereals based diets. *Czech J. Anim. Sci.*, 49,(12): 542–548.
- Preston, C.M., McCracken, R.J. and McAllister, A. 2000.** Effect of diet form and enzyme supplementation on growth, efficiency and energy utilization of wheat based diets for broilers. *Br. Poult. Sci.*, 41: 324-331.
- Ptak, A. ; Bedford, M.; Świątkiewicz, S.; Żyła, K. and Józefiak, D. 2015.** Phytase modulates ileal microbiota and enhances growth performance of the broiler chickens. 20th European Symposium on Poultry Nutrition | 24–27 August 2015 | Prague, Czech Republic.
- Qian, H., Kornegay, E. T. and Denbow, D. M. 1997.** Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. *Poult. Sci.* 76:37–46.
- Ravindran V., Bryden W.L. and Kornegay E.T. 1995.** Phytates: occurrence, bioavailability and implications in poultry nutrition. *Poultry and Avian Biology Reviews*, 6 :125-143.
- Ravindran V., Cabahug S., Ravindran G., Selle P.H., Bryden W.L. 2000.** Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. *Brit. Poult. Sci.*, 41, 193–200.
- Ravindran, V., Bryden, W. L. and Kornegay, E. T. 1995a.** Phytates: Occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.* 6:125–143.
- Reisenfeld, A., (1972). Metatarsal robusticity in bipedal rats. *Am.J. Phys. Anthropol.*, 40: 229–234.
- Salem, F., El-Alaily; H., El-Medany, N. and Abd El-Galil, K.2003** Improving phosphorus utilization in broiler chick diets to minimize phosphorus pollution. *Egypt. Poult. Sci.*, 23:201-218.
- Salari, S., Kermanshahi, H. and Moghaddam, H. 2006.** Effect of sodium bentonite and comparison of pellet vs. mash on performance of broiler chickens. *Int. J. Poult. Sci.*, 5 (1): 31-34.
- SAS Statistical Analysis System2001.** User's Guide Version 8.2, Cary NC. USA.
- Savory, C. J. 1974.** Effect of group size on the feeding behaviour and growth of chicks. *Br. Poult. Sci.*, 16 (1-6): 343-350.
- Sebastian, S., Touchburn, S. P., Chavez, E. R. and Lague, P. C. 1996.** The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. *Poult. Sci.* 75:729–736.
- Selle P.H., and Ravindran V. 2007.** Microbial phytase in poultry nutrition. A review. *Animal Feed Science and Technology*, 35:1-41.
- Shahir , M.H., Haghparast , M.A., Taheri, H.R. and Baradaran, N.2015.** Adaptation to low phosphorus diets in broilers. 20th European Symposium on Poultry Nutrition | 24–27 August 2015 | Prague, Czech Republic.
- Silversides F.G., Scott T.A. and Bedford M.R. 2004.** The effect of phytase enzyme and level on nutrient extraction by broilers. *Poultry Science*, 83:985-989.
- Slominski B.A., Davie, T., Nyachoti, M.C., and Jones, O. 2007.** Heat stability

- of endogenous and microbial phytase during feed pelleting. *Livestock Science*.109: 244-246.
- Sulabo, R. C., Jones, C. K., Tokach, M. D., Goodband, R. D., Dritz, S. S., Campbell, D. R., Ratliff, B. W., DeRouchey, J. M. and Nelssen, J. L. 2011.** Factors affecting storage stability of various commercial phytase sources. *J. Anim. Sci.* 89:4262–4271.
- Takemasa, M., and Hijikuro, S. 1983.** Effects of pelleting on the utilization of phosphorus for chicks. *Jpn. Poult. Sci.* 20:330–336.
- Ullah, A.H.J. and Mullaney, E.J. 1996.** Disulfide bonds are necessary for structure and activity in *Aspergillus ficuum* phytase. *Biochem. Biophys. Res. Commun.*, 227: 311-317.
- Underwood E.J. 1981.** The Mineral Nutrition of Livestock. 2nd Ed. Commonwealth Agricultural Bureau X.
- Viveros, A., Brenes, A., Arija, I. and Centeno, C. 2002.** Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. *Poult. Sci.*, 81(8): 1172-1183.
- Walk, C.L.; Wyatt, C.L.; Upton, R. and McElroy, A.P. 2011.** Effect of diet and phytase on the performance and tibia ash of broilers exposed to a live coccidia oocyst vaccine. *The journal of applied poultry science.* 20(2):153-161.
- Wiernusz, C. 2012.** Using pellet quality to improve broiler growth rate, feed conversion. [http:// www.wattagent.com/](http://www.wattagent.com/)
- Using- pellet- quality -to -improve- broiler- growth -rate- feed- conversion. <http://www.wattagent.com/>
- Wilkinson , S.J. Walk , C. L., Bedford , M. R. and Cowieson, A. J.2013.** Influence of conditioning temperature on the postpellet recovery and efficacy of 2 microbial phytases for broiler chicks. *J. Appl. Poult. Res.* 22 :308–313.
- Woyengo ,T. A.; Adewale E. , Owusu-Asiedu , A., Wilhelm G. , Simmins P.H. and Charles M. N. 2010.** Performance and nutrient utilization responses in broilers fed phytase supplemented mash or pelleted corn-soybean meal-based diets. *J. Poult.Sci.*, 47: 310-315.
- Woyengo T.A., Guenter W., Sands J.S., Nyachoti C.M. and Mirza M.A. 2008.** Nutrient utilisation and performance responses of broilers fed a wheat-based diet supplemented with phytase and xylanase alone or in combination. *Animal Feed Science and Technology*,146: 113-123.
- Zohair, G., Maktari, G. A. and Amer, M. M. 2012.** A comparative effect of mash and pellet feed on broiler performance and ascites at high altitude (field study). *Global Vet.*, 9 (2): 154-159.
- Zyla K., Koreleski J., Swiatkiewicz S., Ledoux D.R., Piironen J. 2001.** Influence of supplemental enzymes on the performance and phosphorus excretion of broilers fed wheat-based diets to 6 weeks of age. *Anim. Feed Sci. Technol.*, 89, 113–118

المخلص العربي

تقييم الكفاءة الحيوية والثبات الحرارى لمصادر مختلفة من انزيم الفيتيز فى العلائق المحببة والناعمة لبدارى التسمين

أميره محمود رفاعى، عبده جاد عبد الله، نادية لطفى رضوان، عبير ربيع خشت، احمد محمد عبد الخالق
معهد بحوث الإنتاج الحيوانى- مركز البحوث الزراعية - الدقى - الجيزة - مصر

استخدم فى هذه الدراسة عدد ٧٨٠ طائر اربور ايكرز غير مجنس عمر يوم حيث قسمت الطيور الى ٢٦ معاملة تجريبية بكل منها ٣٠ طائر. صممت هذه الدراسة فى تجربتين لمقارنة ٣ مصادر لانزيم الفيتيز مختلفة فى المصدر الميكروبي المخلق لها وثباتها حراريا على الاداء الانتاجى وترسيب المعادن فى العظم لبدارى التسمين. فى التجربة الاولى: تغذت الطيور على علائق منخفضة فى محتواها من الفوسفور المتاح بمعدل ٤٠% عن احتياجات السلالة حيث تغذت على ٣,٠٠, ٢٧,٠% فوسفور متاح خلال مرحلتى البادى والنامى على التوالى واضيف اليها أحد انزيمات الفيتيز الاتية : فيتيز I (مخلق من *Schizosaccharomyces pombe*)، فيتيز II (مخلق من *Trichoderma reesei*)، فيتيز III (مخلق من *Pichia pastoris yeast*) كلا فى ٣ مستويات (٠, ٥٠٠, ٧٥٠ وحدة انزيم/كجم علف) مع ٢ شكل للعلف (ناعم ومحبيب) فى تجربة عاملية (٣×٣×٢). وفى تجربة موازية ، مع خفض مستوى الفوسفور المتاح الى ٢٠, ٤٠% عن احتياجات السلالة مع انزيم الفيتيز الاكثر ثباتا حراريا ، من خلال التجربة الاولى، بثلاث مستويات (٠, ٥٠٠, ٧٥٠ وحدة انزيم/كجم علف) و ٢ شكل للعلف : ناعم ومحبيب (التجربة الثانية) فى تجربة عاملية ٢×٣×٢. فى كلا التجريبتين استخدم ٢ مجموعة مقارنة تغذت على احتياجات السلالة من الفوسفور المتاح (٠, ٥, ٤٥, ٠% خلال البدى والنامى على التوالى) فى كلا شكلى العلف ناعم ومحبيب حيث استخدمت هذه المعاملات للمقارنة. وكانت أهم النتائج المتحصل عليها هى:

- ١- نستخلص من هذه التجربة وجود اختلاف فى الثبات الحرارى لمصادر الفيتيز المستخدمة باختلاف المصدر الميكروبي.
 - ٢- سجلت الطيور المغذاة على علائق مضاف اليها فيتيز I أو فيتيز III زيادة معنوية فى وزن الجسم المكتسب ونسب الذبيحة مقارنة بالاخري المضاف اليها فيتيز II.
 - ٣- سجلت مجموعة الطيور المغذاة على علائق محببة تحسنا معنويا فى مقاييس النمو مقارنة بالاخري المغذاة على علائق ناعمة.
- ويمكن ان نستخلص من النتائج ان فيتيز I (مخلق من *Schizosaccharomyces pombe*) هو الاكثر ثباتا حراريا خلال عملية التكميع بينما وجد ان الطيور المغذاة على علائق بها -٤٠% فوسفور متاح ومضاف اليها أى مصدر أو مستوى من انزيم الفيتيز لم تصل الى قيم مجموعة المقارنة فى مقاييس النمو وترسيب المعادن فى العظم. بالنسبة للتجربة الثانية:
- ١- سجلت الطيور المغذاة على علائق بها -٢٠% فوسفور متاح تحسنا معنويا فى كفاءة التحويل الغذائى بمعدل ٤١,٠% خلال فترة التجربة كاملة وكذلك زيادة معنوية فى بلازما الدم للفوسفور والكالسيوم بمعدلات ٣٤,٨%، ٢٧,٧% على التوالى مقارنة بالاخري المغذاة على علائق بها -٤٠% فوسفور متاح.
 - ٢- سجلت الطيور المغذاة على علائق مضاف اليها فيتيز I بمعدل ٥٠٠ أو ٧٥٠ وحدة انزيم/كجم علف زيادة معنوية فى وزن الجسم الحى ونسبة الذبيحة وتحسن كفاءة التحويل الغذائى خلال فترة التجربة .
 - ٣- سجلت الطيور المغذاة على علائق محببة تحسنا معنويا فى الكفاءة التحويلية خلال فترة التجربة بمعدل ٤١,٠% مقارنة بالاخري التى تغذت على علائق ناعمة .
- وعليه يمكن التوصية بان اضافة فيتيز I (مخلق من *Schizosaccharomyces pombe*) بمعدل ٥٠٠ وحدة انزيم/كجم علف للعليقة التى تحتوى على -٢٠% فوسفور متاح سواء فى صورة ناعمة او محببة حسنت من النمو وترسيب المعادن فى العظم.