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# EFFECT OF MICROBIAL PHYTASE ENZYME SUPPLEMENTATION ON GROWTH PERFORMANCE AND BONE MINERALIZATION OF BROILER CHICKS REARED UNDER MILD HEAT STRESS

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## SUMMARY

The present study was conducted to evaluate the effect of using microbial phytase enzyme (Natuphos) on performance and bone mineralization grade (BMG) of broiler chicks fed corn-soybean meal diets from hatch to 35 d of age and reared under mild heat stress. A 2x2x2 factorial treatment arrangement was used: Two levels of ambient temperatures, 20 $^0$C (neutral temperature =NT) and 30 $^0$C (high temperature = HT), two levels of non-phytate phosphorus (NPP), 0.45 % (control) and 0.34% and two levels of phytase enzyme (PE), 0 and 500 FTU phytase /kg diet. The Ca: NPP ratio was constant for all treatments being 2.22:1. Two hundred forty one-d old unsexed Hypro broiler chicks were distributed equally into 8 experimental treatments in 2 replicates of 15 birds each. Chicks were reared in separated floor pens in closed house (15-bird/ m$^2$). At the end of the experiment, 6 birds from each pen, 3 from each sex were slaughtered for BMG measurement. Several parameters were measured to assess broiler performance and bone mineralization grade at different periods, i.e. body weight (BW) and gain (BWG), feed consumption (FC) and feed conversion (FC/BWG), leg abnormality, mortality rate and BMG at different periods. Temperature had a significant effect on the body weight of broiler. Chicks in the NT (20 $^0$C) had a significantly heavier BW and BWG at studied periods compared to those in the HT (30 $^0$C). Absolute FC, (FC/BWG) and BMG showed the same trend. Best results of BW and BWG and (FC/BWG) were obtained with decreasing NPP levels (0.34%) compared to that fed (0.45%) indicating a potential for improving the utilization of dietary Phytate-P. On the other hand, the highest BMG was observed when chicks fed on diets with 0.45% NPP. The addition of PE at 500 FTU increased significantly (P<0.01) BW and BWG, (FC/BWG) and BMG when compared to the chicks fed the experimental diet with no phytase supplement. Significant interaction effects (TxNPPxPE) were observed on studied criteria BW, BWG, and (FC/BWG) from 0-5 wks of age and BMG at 35 d of age. The total mortality rate was 1.25% during the total experimental period (0-5 wks) without any obvious differences among treatments. This study indicated that feeding broiler chick 500 FTU PE /kg diet with low level of NPP (0.34%) and constant Ca: NPP ratio (2.22:1) resulted in improving chick's performance raised under mild heat stress (30 $^0$C).

**Keywords**: *phytase enzyme, heat stress, growth performance, bone mineralization, broiler chicks.*

## INTRODUCTION

In developing countries, the demand for importing corn grains and soybean meal as feed ingredients for poultry industry has been increasing under the intensive production systems. More than

60 % of phosphorus (P) in corn and soybean meal is in the form of phytate-phosphorus, PP (Nelson *et al.*, 1968; and Reddy *et al.*, 1982). This phytate-P (PP) has low P availability and the bioavailability experiments estimated that P availability in corn grains and soybean meal for poultry ranged from 10 to 30 % (Nelson 1967; Calvert *et al.*, 1978 and Jongbloed and Kemme 1990). The remaining 70-90% is in a complex form called " phytin –P, in which with the absence of enzyme phytase in the digestive tract of poultry it cannot be utilized and therefore excreted in poultry excreta. On the other hand, ruminants can utilize PP effectively due to the production of phytase by the microorganisms of the rumen.

To meet the optimum P requirement of poultry, specially broiler chicks, it is important to add inorganic-P (non-phytate phosphorus = NPP) sources (such as dicalcium phosphate, defluorinated phosphate, bone meal.....etc) (Abel *et al.*, 1982; Gunther *et al.*, 1982 and Hermes *et al.*, 1983).

It is well known that large amount of N and P are present in poultry manure. Excretion of P and N through poultry wastes has received a considerable degree of attention due to their potential in polluting the environment. Nitrogen excretion can be reduced considerably through using low protein levels with amino acids supplemental diets (Kesbavarz 1996). In respect of P, satisfactory performance according to the NRC recommendation (1994) can be reached by P phase feeding NPP: of 0.45; 0.35 and 0.30 % from hatching to 3; 3 to 6 and 6 to 8 weeks of age during the growth periods of broiler chicks, respectively. Several reports concluded that P phase feeding can be implemented successfully as a means of reducing both feed costs and P pollution (Sauveur 1978; Yoshida and Hoshii 1982 and Tortuero and Tardon 1983).

In the last few years, more recent and successful approach for reducing the P content of broiler diets was achieved by using microbial phytase in the diet. It means that microbial phytase from the mold Aspergillus-Ficuum is effective in releasing a significant portion of the PP present in corn grains and soybean meal and making it available to broiler chickens and growing turkeys (Simons *et al.*, 1990; Schoner *et al.*, 1991;Broz *et al.*, 1994; Denbow *et al.*, 1995; Sebastian *et al.*, 1996; and Atia *et al.*, 2000). Many investigators (Ravindran *et al.*, 1995; Yi *et al.*, 1996; Sebastian *et al.*, 1996; Atia *et al.*, 2000; El Deeb *et al.*, 2000) reported that phytase from Natuphos as a commercial enzyme preparation, resulted in improved availability of PP. BASF-information's (2000) reported that Natuphos can now be used as a feed ingredient with assigned nutrient specification in least cost formulation, and in the USA, where corn and soybean meal diets are the standard ingredients, Natuphos helps to save up to US $ 2.70 / metric ton of broiler feed.

Productive performance (BW, BWG, FC and FC/BWG) and bone mineralization (toe ash and tibia ash percentages) are sensitive indicators for biological assessing the efficiency of PE in Natuphos for broiler chickens and growing turkeys fed commercial diets based on corn grains and soybean meal (Kornegay *et al.*, 1996 and Atia *et al.*, 2000). The results of trials concerning the suitable dose of microbial phytase have shown a linear dependence on the dose up to 500 FTU/ kg of feed. The response then tends to flatten to varying degrees at higher doses (BASF-informations 2000).

The lack of information and the contradictions concerning the efficacy of phytase on the performance of broiler chickens reared under heat stress indicate the need for more investigation. Therefore, the objective of this study was to determine the response of broiler chickens reared under mild heat stress from hatching to 35 days of age to microbial phytase added to corn/soybean meal based diets.

## MATERIALS AND METHODS

The present experiment was carried out at the Poultry Research Station, Department of Animal Production and Breeding, College of Agriculture and Veterinary Medicine, King Saud University (Al-Qassim branch), Saudi Arabia. The experiment started in October 2000 and lasted for 5 weeks. Total of 240 one–day old unsexed Hypro broiler chicks were obtained from a commercial hatchery. Chicks were wing banded and reared over 5 weeks in separated floor pens (15 birds / m2 ) in closed house. The house consists of 2 rooms and 8 pens per room. Chicks were randomly distributed into two temperature groups. The initial temperature was 32 0C during the 1st week then it was gradually reduced by 4 0C / week to reach 20 0C after 4 weeks and was maintained at this degree in this room ,which served as a neutral temperature (NT), while in the other room, which served as high temperature (HT), chicks were reared during the whole experimental period (from hatching to 35 days of age) under high temperature 30 0C. Ventilation, air condition and heating in each of the 2 rooms was controlled by a Dicam FSC2.2M master unit (Farm Energy and Control Services Ltd, (FARMEX), Pinewood, Reading RG 303VR, United Kingdom). The unit contains an integrate processor, process times, and serial parts. Each room has a dedicated temperature sensor leading to the Dicam unit. The unit controlled the temperature to preset limits, and inducted preset minimum ventilation rates, which were increased with the age of the bird. Routine adjustments to the individual room requirements were carried out on a daily basis by reconfiguring the system at the Master unit, the true air temperature for each experimental room was recorded automatically/hour/day using the same unit. Average relative humidity during the experiment was 50% and 40% in the neutral and high temperatures, respectively Lighting program was (23L+1D) during the whole experimental period. The experimental rooms were provided with wood shaving litter, suitable number of hanging feeders and automatic waterers.

The basal experimental diets as shown in Table (1) were formulated to meet the nutrient requirements of broiler chicks based on the recommendation of NRC (1994) and containing 23 % crude protein and providing 13.6 MJ ME /kg diet. The experimental diets were formulated using corn grains and soybean meal to contain: 1) NPP and Ca levels of 0.45 % and 1.0 % respectively (control); 2) NPP was reduced to 0.34 % and Ca was reduced to 0.75 %. These dietary levels of NPP = 0.34 % and 0.75 % Ca were formulated below the current NRC, 1994 (75 % of NRC, 1994) and were designed according to Yi *et al*., (1996) to ensure a maximum response to PE supplemantion. The Ca: NPP ratio was maintained at 2.22:1 in all experimental diets. Because PP was supplied only from corn grains (50%) and soybean meal (38.45%) so, the dietary percentage of PP (0.25%) was similar in all experimental diets. In the same order, the diets were formulated primarily from corn/soybean meal in which they contain negligible amounts of indigenous phytase and similar to diets used commercially. The two experimental diets were fed with or without enzyme supplementation, using Natuphos as a commercial enzyme preparation at the level of 500 FTU / kg diet. A unit of phytase is defined as the quantity of enzyme that liberates 1 μ mol of inorganic-P/min from 5.1-mM sodium phytate at pH 5.5 and 37 0C. Natuphos enzyme preparation was provided by BASF-AG, D-67056 Ludwigshafen, Germany. Finely ground screened corn was used to facilitate the enzyme–supplementation pre mixing for the experimental treatments, then mixed gradually with the other feed ingredients using a small food – type mixer. Feed and water were supplied ad- libitum.

Criteria used to measure phytase enzyme response were: individual live body weight and body weight gain, group feed consumption and feed conversion (feed/gain), leg abnormality and mortality rate. To estimate bone mineralization grade, at the end of the experiment (35 days of age) 6 birds from each pen, 3 from each sex were randomly selected and sacrificed. Right tibiae were cleaned of adhering tissues. Fresh right tibiae were dried, extracted with ether, dried and weighed again. The fat-free dry bones were ashed in a muffle furnace at 550 0C. The bone mineralization grade was calculated according to Brune and Gunther (1959). Chemical composition of the experimental diets was determined according to AOAC (1990)

To estimate bone mineralization grade, at the end of the experiment (35 days of age) 6 birds from each pen, 3 from each sex were randomly selected according to shape and size of comb and sexual organs. Birds were sacrificed and right tibiac were cleaned of adhering tissues. Fresh right tibiae were dried, extracted with ether, dried and weighed again. The fat-free dry bones were ashed in a muffle furnace at 550 0C. The bone mineralization grade wea calculated according to Brune and Gunther (1959). Chemical composition of the experimental diets was determined according to AOAC (1990) .

***Statistical analysis***

Data (bodyweights, gains, feed consumption and feed conversion) were subjected to three-way analysis of variance concerning temperature, NPP level and enzyme level (main effects) and their interaction by using the General Linear Models (GLM) procedure of SAS User's Guide, (1996) according to the following linear model:

Yijklmn = μ + Gi + Aj +Bk +Cl + Dm +ABjk + AC jl

Data were analyzed using GLM procedure of SAS program (SAS, 1996). Body weights and gains were analyzed by adapting the following linear model:

Yijklmn = μ + Rk + Cj + Dm +BCDklm + eiklmn (Model 1)

Where: μ = overall mean; Bk = the effect of kth temperature (1= 30 0C , 2 = 20 0C);
Cl = the effect of lth NPP level (1= 0.45 % , 2 = 0.34 %);
Dm = the effect of mth enzyme level (1= 0 U/kg , 2 = 500 U/kg),
BCDklm = three–order interaction of subscripted factors;
eiklmn = a random error.

Data of bone mineralization grade were analyzed using the following linear model:

Yiklmn = μ+ AJ + Bk + Cl + Dm + BCDklm + eiklm (Model 2)

Where AJ= the effect of jth sex

All other abbreviations of this model were defined previously in model 1. The effect of four-order interactions (AxBxCxD) from the model of analysis was deleted. Duncan, s new multiple range test (Duncan, 1955) was used to test mean differences, when a significant value was observed

**Table (1): Components and calculated composition of the experimental basal diets from hatch to 5 weeks of age.**

| Ingredients (%) | Non phytate phosphorus (NPP) [1] % | |
|---|---|---|
| | 0.45% (control) | 0.34% |
| Ground yellow corn | 50.000 | 50.000 |
| Soybean meal (48%) | 38.450 | 38.450 |
| Animal fat | 7.785 | 7.865 |
| Di-calcium phosphate(2) | 1.755 | 1.140 |
| Limestone (3) | 1.330 | 1.020 |
| Salt | 0.300 | 0.300 |
| DL-Methionine (99%) | 0.200 | 0.200 |
| Broiler mineral premix(4) | 0.050 | 0.050 |
| Broiler vitamin premix(5) | 0.050 | 0.050 |
| Choline chloride | 0.080 | 0.080 |
| Sand | ----- | 0.845 |
| Total | 100.000 | 100.000 |
| **Calculated composition (%):** | | |
| ME (MJ / kg) | 13.546 | 13.571 |
| Crude protein | 23.050 | 23.050 |
| Calcium (Ca) | 1.005 | 0.752 |
| Total phosphorus (TP) | 0.707 | 0.592 |
| Non-phytate phosphorus (NPP) | 0.453 | 0.338 |
| Ca : NPP | 2.220 | 2.220 |

**(1) Non-Phytate phosphorus levels as percentage of NRC (1994). 0.45% = 100% and 0.34 % = 75 %.**

**(2) Di-calcium phosphate: Calcium 22% and phosphorus 18.7%.**

**(3) Limestone: Calcium 38.0%.**

**(4) Broiler mineral premix (mg/kg of premix): 70000 Iron; 100000 Zinc; 40000 Copper; 160000 Manganese; 700 Iodine; 300 Selenium and 800 Cobalt.**

**(5) Broiler vitamin premix (per kg of premix): 25 million IU Vit. A; 5 million IU Vit. $D_3$ ; 110 g Vit E acetate (alfa – tocopherol 91%); 2.5 g Vit. $B_1$; 11 g Vit. $B_2$ ; 18 g D- Pantothenic acid ; 120 mg Biotin ; 36 mg Vit. $B_{12}$; 62 g Vit PP; 1.8 g Folic acid; 5 g Vit.$K_3$ and 200 g BHT.**

**Since the phytate--P was supplied only from corn and soybean meal, the dietary content of phytate--P was similar in all experimental diets = 0.254%.**

**Table (2): Least square means (± SE) of growth performance of broiler chicks as affected by heat temperature (T), level of non- phytate phosphorus (NPP) and phytase enzyme supplementation (PE) from hatch to 5 weeks of age.**

| Traits | Heat Temperature (T °C) | | Non-Phytate Phosphorus (NPP %) | | Phytase Enzyme (PE FTU/kg) | | ± SE | RSD |
|---|---|---|---|---|---|---|---|---|
| | 30 °C | 20 °C | 0.34 % | 0.45% | --- | 500FTU/kg | | |
| **Live body weight (g)** | | | | | | | | |
| At 2 wks | 293.8$^b$ | 320.2$^a$ | 306.9 | 307.4 | 300.4$^b$ | 313.5$^a$ | 4.3 | 45.4 |
| At 4 wks | 975.6$^b$ | 1106.8$^a$ | 1037.3 | 1050.9 | 1043.0 | 1045.3 | 11.0 | 115.6 |
| At 5 wks | 1338.2$^b$ | 1558.3$^a$ | 1517.4$^a$ | 1443.0$^b$ | 1468.9 | 1491.5 | 13.3 | 140.1 |
| **Daily weight gain (g)** | | | | | | | | |
| 0 – 2 wks | 18.3$^b$ | 20.1$^a$ | 19.2 | 19.1 | 18.7$^b$ | 19.6$^a$ | 0.4 | 3.2 |
| 2 - 4 wks | 48.7$^b$ | 56.2$^a$ | 52.2 | 53.1 | 52.7 | 52.2 | 0.5 | 5.9 |
| 4 - 5 wks | 51.8$^b$ | 64.5$^a$ | 66.6$^a$ | 57.9$^b$ | 60.1$^b$ | 63.2$^a$ | 0.7 | 7.6 |
| 0 – 5 wks | 37.2$^b$ | 43.4$^a$ | 42.2$^a$ | 40.1$^b$ | 40.6 | 41.4 | 0.4 | 4.0 |

**RSD: Residual standard deviation.**

**a,b values having different superscripts within each row differ significantly($P<0.05$).**

## RESULTS AND DISCUSSION

***Growth performance and feed conversion***

***Temperature (T):***

Growth performance data of broiler chicks as affected by heat temperature (T), level of non-phytate phosphorus (NPP) and phytase enzyme supplementation (PE) are shown in Table (2). The initial BW at hatching day did not differ among the experimental groups and the overall mean was 38.6 g. In general, high ambient temperature of 30 0C (with a maximum deviation of ±1 0C) led to a high significant (P<0.001) reduction in BW compared to those raised at the neutral ambient temperature of 20 0C. The data of BWG (g/d) as shown in Table (2) confirmed this trend, since chicks raised under HT gained less weight than those kept under NT and the corresponding values were: 18.3, 48.7, 51.8, 37.2 versus 20.1, 56.2, 64.5, 43.4 g/d, during 0-2, 2-4, 4-5 and 0-5 weeks of age, respectively. This means that depression in growth performance due to the effect of HT was more pronounced as the age of bird increased, and the younger broiler chicks have more tolerance for HT and more tolerant to heat stress than the older ones. The explanation to that has been reviewed by Teeter (1995), who reported that commercial broilers are particularly susceptible to heat stress, because metabolic heat production increases with age and growth rate, while heat dissipation capacity does not. Many investigators reported that HT has adverse effects on productive performance of broiler chicks (Hussein 1996; Alleman and Leclercq 1997; Al-Homidan *et al.*, (1997 and 1998); Mebta and Sbingari 1999; Al-Homidan (2000 and2001); and Al-Batshan and Hussein 2001).

Data in Table (3) indicated that daily feed consumption decreased as ambient temperature increased and the difference between treatments was significant (P<0.05) during the studied periods. The reduction was more pronounced by older birds especially during the last three weeks of age and the corresponding figures were 4.1, 11.9 and 13.6 % during 0-2, 2-4 and 4-5 weeks of age, respectively. Dale and Fuller (1980) suggested that only 63 % of growth depression due to heat stress is directly related to reduction in feed intake. Under HT, birds try to reduce energy metabolism and protect themselves through starvation (eating less feed to satisfy energy requirement). With starvation, fewer nutrients are available to the body, which is reflected in reduction of BWG. Yousef (1985) concluded that the poor growth performance was mainly due to decrease in feed intake, which leads to less protein biosynthesis and/or less fat deposition. Moreover, the HT reflected physiological changes and reactions on the bird such as an increase in body temperature, respiration rate and heart impulse, in which it consumes more energy and accordingly the remaining net energy for growth are decreased. Feed conversion showed that birds raised under NT were more efficient in converting their food into gain compared with those kept under HT (Table, 3), and difference due to T was significant ((P<0.05) during the studied periods. Other investigators reported similar observations (Teeter 1995; Hussein 1996; Mebta and Sbingari, 1999; Al-Homidan *et al.*, (1997and 1998); Al-Homidan (2000 and 2001); Al-Batshan and Hussein 2001). Contrary to that, Wilson *et al.*, (1980) with poultry (White Peking ducks) and Hermes *et al.*, (1999) with growing rabbits, found that under HT the flow rate of the digesta in the gut decreased that allow the nutrients to act with the digestive enzymes for longer time, which can lead to a better feed utilization.

Non-phytate phosphorus (NPP) supplementation:

Chicks fed the experimental diets containing 0.34% and 0.45% NPP (available-P) without enzyme supplementation showed in most cases no significant differences in live body weight and weight gain (except at 5 wks of age and the period 0-5 wks of age), as shown in Table (2). Till 4 weeks of age, birds receiving 0.45 % NPP were heavier than birds receiving 0.34 % NPP (1050.9 versus 1037.3 g), but the difference failed to be significant. This lower body weight (14g) was due to the deficiency of P in birds fed 0.34% NPP, which is much below (less 33%) the recommended level of 0.45 % NPP for broilers at 0-3 wks of age (NRC, 1994). Contrary to that, after 4 wk of age, chicks fed the lower level (0.34% NPP) reflected a significant ($p<0.001$) increase in body weight and body weight gain compared with the higher level (0.45 % NPP). This confirms the previous recommendations of NRC (1994), indicating that the physiological requirements to obtain satisfactory performance of broiler chicks can be obtained by phase feeding dietary NPP of 0.45% and 0.35 % from 0 to 3 and 3 to 6 wks of age, respectively. In the same order, these results were in accordance with previous observation reported by Sohail and Roland (1999).

Data of feed consumption (g/d) and feed conversion (FC/BWG) are given in Table (3). In most cases, the results of FC and FC/BWG showed inconsistent trend and the differences were significant during 2-4 wks of age and during the whole experimental period (0-5 wks of age). Generally, the better FC/BWG ($P<0.001$) was obtained when birds were fed the diet supplemented with 0.34 % NPP compared with those fed 0.45% NPP (1.65 versus 1.70) during the whole experimental period (0-5wks of age).

***Phytase enzyme supplementation (PE):***

The effects of phytase enzyme supplementation on growth performance are summarized in Table (2). The results revealed that birds fed the experimental diet containing corn plus soybean supplemented with enzyme showed higher body weight and gain in all studies periods and the improvement in body weight gain was 4.8 % and 5.2 % during 0-2 and 4-5 wks of age compared with those fed the diet without enzyme. Treatment effect on BWG was significant ($p<0.05$) at 0-2 and 4-5 wks. In the same order, the data of daily FC and FC/BWG as shown in (Table 3) indicated the same trend. In spite of daily FC in most cases showed relatively the same figures and no significant differences between treatments, the dietary PE supplementation reflected significant ($p<0.05$) positive response on FC/BWG in all studied periods (except 2-4 wks of age). These results showed the positive effects of microbial phytase supplementation on growth and FC/BWG as indications of nutrient utilization by storage form of both phosphate and inositol in almost all seeds (Saylor 2000). Approximately 66% of P in corn and 61% of P in soybean meal is in the form of phytic acid (Nelson *et al.*, 1968). The inability of young broiler chicks to utilize phytic acid has been clearly demonstrated in this study by a slower growth rate and bad FC/BWG. Similar observations of improving body weight with phytase supplementation have been reported for broiler chickens (Simons *et al.*, 1990; Schoner *et al.*, 1991;Broz *et al.*, 1994; Denbow *et al.*, 1995; Ravindran *et al.*, 1995; Sebastian *et al.*, 1996; Yi *et al.*, 1996; El Deeb *et al.*, 2000 and BASF-informations 2000). Phytase enzyme sources with appropriate

**Table (3): Least square means (± SE) of feed consumption (g/bird) and feed conversion (feed/ gain) of broiler chicks as affected by heat temperature (T), level of non-phytate phosphorus (NPP) and phytase enzyme supplementation (PE) from hatch to 5 weeks of age.**

| Traits | Heat Temperature (T °C) | | Non-Phytate Phosphorus (NPP %) | | Phytase Enzyme (PE FTU/kg) | | ± SE | RSD |
|---|---|---|---|---|---|---|---|---|
| | 30 °C | 20 °C | 0.34 % | 0.45% | --- | 500 FTU/kg | | |
| **Feed consumption (FC)** | | | | | | | | |
| 0-2 wk | 26.7 | 27.8 | 27.4 | 27.1 | 27.4 | 27.1 | 0.7 | 1.9 |
| 2-4 wks | $80.9^{b}$ | $90.5^{a}$ | 84.1 | 87.4 | 86.7 | 84.8 | 1.3 | 3.6 |
| 4-5 wks | $101.1^{b}$ | $114.8^{a}$ | $117.8^{a}$ | $111.2^{b}$ | 114.0 | 114.9 | 0.5 | 1.4 |
| 0-5 wks | $65.5^{b}$ | $70.7^{a}$ | 68.8 | 68.3 | 68.8 | 68.2 | 0.7 | 2.0 |
| **Feed conversion (FC/BWG)** | | | | | | | | |
| 0 – 2 wks | $1.46^{a}$ | $1.38^{b}$ | 1.45 | 1.40 | $1.46^{a}$ | $1.38^{b}$ | 0.02 | 0.06 |
| 2 - 4 wks | $1.66^{a}$ | $1.61^{b}$ | $1.60^{b}$ | $1.67^{a}$ | 1.64 | 1.62 | 0.01 | 0.02 |
| 4 - 5 wks | $1.95^{a}$ | $1.78^{b}$ | 1.77 | 1.70 | $1.79^{a}$ | $1.69^{b}$ | 0.03 | 0.08 |
| 0 – 5 wks | $1.76^{a}$ | $1.63^{b}$ | $1.65^{a}$ | $1.70^{b}$ | $1.69^{a}$ | $1.65^{b}$ | 0.01 | 0.02 |

**RSD: Residual standard deviation.**

**a,b Values having different superscripts within each row differ significantly($P<0.05$).**

**Table (4) : Least square means (± SE) of bone mineralization grade of right tibia of broiler chicks as affected by heat temperature (T), level of non-phytate phosphorus (NPP) and phytase enzyme supplementation (PE) and sex (S) at 5 weeks of age.**

| Traits | Heat Temperature (T°C) | | Non-phytate phosphorus (NPP%) | | Phytase Enzyme (PE FTU/ kg) | | Ssex | | RSD | ± SE |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.34% | 20 °C | --- | 0.45% | 30 °C | 500 FTU/kg | M (1) | F (2) | | |
| Fat free DM weight (g) | $5.65^{b}$ | $6.26^{a}$ | $5.63^{b}$ | $6.30^{a}$ | 5.97 | 6.10 | $6.62^{a}$ | $6.29^{b}$ | 0.09 | 0.56 |
| Ash weight (g) | $2.21^{b}$ | $2.49^{a}$ | $2.10^{b}$ | $2.70^{a}$ | $2.28^{b}$ | $2.51^{a}$ | $2.64^{a}$ | $2.46^{b}$ | 0.04 | 0.23 |
| Organic matter weight (g) | $3.44^{b}$ | $3.77^{a}$ | $3.53^{b}$ | $3.60^{a}$ | $3.69^{b}$ | $3.59^{a}$ | $3.98^{a}$ | $3.77^{b}$ | 0.05 | 0.33 |
| Bone mineralization Grade (3) | $0.642^{b}$ | $0.660^{a}$ | $0.595^{b}$ | $0.750^{a}$ | $0.618^{b}$ | $0.699^{a}$ | $0.663^{a}$ | $0.637^{b}$ | 0.002 | 0.01 |
| Ash in DM weight (%) | $39.04^{b}$ | $40.70^{a}$ | $36.05^{b}$ | $42.10^{a}$ | $38.10^{b}$ | $41.68^{a}$ | $39.89^{a}$ | $38.87^{b}$ | 0.07 | 0.39 |

**(1) Male (2) Female**

**3) Bone mineralization grade = Ash weight(g) / Organic matter weight, (g)**

**RSD: Residual standard deviation.**

**a,b Values having different superscripts within each row differ significantly ($P< 0.05$)**

activities have been used successfully to overcome the adverse effect of high phytic acid and to increase the nutritional values of feed ingredients such as cereals and legumes (corn, wheat, sorghum, barely, soybean meal, cottonseed meal...etc) for poultry. This response is distinct in young birds and beneficial response has sometime also been shown for adult birds. Kesbavarz (1996) reported that a laying corn-soybean diet might not need any supplemental inorganic-P if microbial phytase is used.

***Bone mineralization grade (BMG):***

The effects of the experimental treatments on calculated bone mineralization grade according to Brune and Gunther (1959) of right tibia at 5 weeks of age are presented in Table (4). High ambient temperature of 30 0C led to a high significant (P<0.001) reduction in ash weight of right tibia (2.21 versus 2.49 g) compared to those raised at the neutral ambient temperature of 20 0C and bone ash content decreased by 11.3%. The calculated BMG (g ash weight/ g organic weight) showed the same trend since differences were significant (P<0.05) due to HT. The reduction in ash weight and calculated BMG could be explained on the basis that heat stress (30 0C) caused a high reduction in FC being 13.6 % during 4-5 wks of age (Table, 3). It was obvious from Table (4) that increasing the dietary level of NPP to 0.45% significantly (p<0.001) increased tibia ash and calculated BMG compared to the diet containing 0.34% NPP. That means when NPP was increased from 0.34% to 0.45%, calculated BMG increased by 26.1%. This increase in BMG was expected, because about 80% of the total body-P and 99% of the total body-Ca are in the bones. It is very well known that poultry bones consist of an organic matter (protein, fat and polysaccharide) as matrix, which becomes mineralized with Ca and P in a 2:1 ratio as hydroxylapatite [3Ca3 (PO4)2 - 2Ca (OH)2 ] according to Brune and Gunther (1959). Although productive performance is an important measure of any dietary changes, when NPP and Ca levels or one of the two in the diet changed, BMG measures are generally more sensitive than other performance factors such as LBW, BWG, FC and FC/BWG (Brune and Gunther 1959; Hermes 1982; Denbow *et al.*, 1995; Sebastian *et al.*, 1996 and Sohail *et al.*, 1999). The results of the present experiment confirmed this fact, in which birds fed the diet supplemented with 0.34% NPP and 0.75 % TP showed the better results for growth at 5 weeks of age but not enough for bone development and mineralization. On the other hand, these findings are in contrast with the results obtained by Ravindran *et al.*, (1995), who found that BWG and toe ash percentage for 3 wks of age were equally or more sensitive for assessment of P availability than tibia ash, and concluded that birds are very sensitive to dietary P because of their characteristic low P storage and fast growth. In the same order, Simons *et al.*, (1990) and Yi *et al.*, (1996) reported that BWG and toe ash percentage were found to be sensitive measurements to evaluate P availability in diets of poultry.

As expected, male chicks showed significantly (P<0-05) higher values in respect to BMG than female, as shown in Table (4). These differences could be explained on the basis that generally, male chicks showed significantly higher values than female in BW, BWG, FC and FC/BWG especially in older age (Al-Homidan *et al.*, 2000).

The data in Table (4) revealed that tibia ash weight (g), the percentage of ash in dry matter weight and the calculated BMG were significantly improved by addition of dietary phytase. These results are in agreement with previous studies with broiler chickens (Broz *et al.*, 1994; Denbow *et al.*, 1995; Sebastian *et al.*, 1996) and growing turkeys (Atia *et al.*, 2000).

The mechanisms of phytase enzyme may affect the metabolism of the bird in different ways: 1)-The utilization of inositol by mono-gastric animals (poultry, pigs.....etc).Phytase catalyses the dephosphorylation of phytic acid by stepwise removal of orthophosphates, resulting in the intermediates inositol mono to penta- phosphate and free myo-inositol , as suggested by Simons *et al.*, (1990). 2)- Increased energy metabolism from fat and it is probably that the high molar ratio of Ca to phytate leads to the formation of insoluble Ca-phytate complex. Therefore contributing to the anti-nutritional effect of phytate on energy availability possible explanation is that Ca-phytate complexes with fatty acids in the gut lumen to form insoluble metallic soaps thereby lowering fat digestibility, as suggested by Simon *et al.*, (1996) and BASF-informations (2000). 3)-Increased of starch digestibility, as suggested by Knuckles and Betschart (1987). 4)- Improved availability of protein. The anti-nutritional effect of phytate on protein digestibility and availability may be due to that protein and amino acids cause a complex bonds with phytate, and thus cannot be absorbed by birds. In the highly acidic stomach region amino acids, in particular lysine, methionine, arginine and histidine are bounds directly to phytate-P creating practically insoluble phytate-protein complexes, as suggested by Simon *et al.*, (1996) and BASF-informations (2000). 5)-The release of minerals (Ca, P, Mn, Zn, Cu, ...etc) from the phytate–mineral complex, as suggested by Sebastian *et al.*, (1996) and BASF-informations (2000).

Interactions between heat temperature (T), level of non-phytate phosphorus (NPP) and phytase enzyme supplementation (PE), (TxNPPxPE interactions):

Growth performance, feed conversion and bone mineralization grade as affected by interactions between T, NPP and PE are summarized in Table (5). The studied criteria, final live body weights and accumulative body weights gain and feed conversion from hatch to 5 wks of age were significantly ($P<0.05$) affected by TxNPPxPE interactions. This indicates that, under the conditions of this experiment, NPP (0.34 and 0.45%) and PE (0 and 500 FTU/kg diet) act jointly under the effect of T (200C and 30 0C) during the experimental period (0-5wks of age) of broiler chicks. In the same order, the BMG of right tibia showed the same trend.

Unfortunately, there are no available published literature dealing with the effect of interactions between T, NPP, and PE on the performance and bone mineralization of broiler chickens and therefore no comparisons were made with results of the present study.

However, there are numerous inconsistent and conflicting findings surrounding the effect of phytase enzyme supplementation on broiler diets. Simon *et al.*, (1996) concluded that successful responses to enzyme supplementation depend on different factors such as: the presence of target substance, dietary P-level, enzyme concentration, enzyme activity, dietary ingredients, anti-nutritional factors, antibiotics, age, environmental conditions ...etc. Since enzymes are proteins, they are susceptible to destruction through heat, pH, and proteolytic enzymes.

***Health condition and mortality rate:***

Under the condition of the present study all chicks appeared healthy, no

**Table (5) : Least square means (± SE) of growth performance bone mineralization grade of broiler chicks as affected by heat temperature (T), level of non-phytate phosphorus (NPP) and phytase enzyme supplementation (PE).**

| Traits | Interaction cell | | | | | | | | Significance |
|---|---|---|---|---|---|---|---|---|---|
| | 30 C° | | | | 20 C° | | | | |
| | 0.34%NPP | | 0.45%NPP | | 0.34%NPP | | 0.45%NPP | | |
| | ------- | 500FT U/kg | ------ | 500FT U/kg | ----- | 500FT U/kg | | 500FT U/kg | |
| LBW | 1458±148 | 1518±215 | 1432±204 | 1388±151 | 1489±187 | 1541±157 | 1462±165 | 1496±193 | * |
| DWG | 40.5±4.2 | 42.3±6.2 | 39.9±5.8 | 38.6±4.3 | 41.4±5.3 | 42.9±4.5 | 40.7±4.7 | 41.7±5.5 | * |
| DFC | 66.4±1.0 | 65.2±3.0 | 65.9±0.8 | 64.4±0.8 | 71.5±3.6 | 71.9±1.5 | 71.4±1.7 | 71.4±0.7 | NS |
| FC/BWG | 1.64±0.02 | 1.55±0.05 | 1.66±0.02 | 1.67±0.01 | 1.73±0.03 | 1.68±0.01 | 1.75±0.24 | 1.71±0.01 | * |
| AW | 2.14±0.46 | 2.45±0.45 | 2.33±0.55 | 1.92±0.21 | 2.25±0.38 | 2.47±0.18 | 2.40±0.35 | 2.83±0.31 | *** |
| OMW | 4.79±0.67 | 3.6±0.51 | 3.60±0.67 | 2.78±0.22 | 3.81±0.61 | 3.71±0.30 | 3.57±0.51 | 4.00±0.44 | *** |
| BMG | 0.56±0.02 | 0.68±0.03 | 0.64±0.03 | 0.69±0.03 | 0.59±0.01 | 0.67±0.02 | 0.67±0.1 | 0.71±0.1 | * |

**Growth performance:**
**LBW= Live body weight,at 5 wks (g) DWG = Daily weight gain, 0-5 wks (g).**
**DFC= Daily feed consumption, 0-5 wks(g) FC/BWG = Feed conversion, 0-5 wks(g).**
**AW= Ash weight of right tibia, at 5 wks (g). OMW = Organic matter weight, at 5 wks (g).**
**Significance: NS + Non- Significant; * = p<0.05; ** = p<0.01; *** = p<0.00**

$$\text{BMG= Bone mineralization grade} = \frac{\text{Ash weight (g)}}{\text{Organic matter weight (g)}}$$

**Significance :**
**NS: Non significant, * = p<0.05; ** p<0.01; *** = p<0.001**

clinical symptoms were observed in relation to leg abnormalities, incidences of rickets. The total mortality was 1.25% (Total dead birds = 6) during the whole experimental period from hatching to 5 wks of age without any clear differences among treatments.

### *Conclusion*

From the results obtained in this study it could be concluded that feeding broiler chicks 500 phytase units FTU /kg diet with low level of NPP (0.34%) and constant Ca: NPP ratio (2.22:1) resulted in improved chick performance and bone mineralization grade in chicks raised under mild heat stress (30 0C).

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## REFERENCES

Abel, HJ.; I. H. Hermes and K. D. Gunther (1982). Evaluation of the intermediary availability and the efficiency of utilization of feed phosphorus in growing chicken. Methodological assumptions. Z. Tierphysiol. Tierernahahrg. U. Futtermittelkde. 48: 154-159.

AL-Batshan, H.A. and E. O. S. Hussein (2001). The effect of total sulfur amino acids on performance and carcass composition of broilers under heat stress. J.King Saud Univ., Agric. Sci., 13: 31-43.

AL-Homidan, A. (2000). Effect of vitamin C addition in water for broilers raised under two different ambient temperatures on growth performance and water consumption. Egypt. Poult. Sci.J., 20: 327-346.

Al-Homidan, A. (2001). The effect of temperature and stocking density on broiler performance and ammonia production. Egypt. Poult. Sci.J. 21: 1121-1137.

AL-Homidan, A.; J.F. Robertson and A.M. Petchey (1997). The effect of temperature, litter and light intensity on ammonia and dust production and broiler performance. Brit. Poult. Sci. 38:S5-S7.

AL-Homidan, A, J.F.Robertson and A.M. Petchey (1998). The effect of environmental factors on ammonia and dust production and broiler performance. Brit. Poult. Sci. 39:S9-S10.

AL-Homidan, A.; Y. AL-Seleem; I. Hermes and B. Ahmed (2000). Growth performance and carcass characteristics of broiler chicks fed different cereal grains supplemented with enzyme preparation. Egypt. J. Nutrition and Feeds 3 (1) 57-69.

Alleman, F. and B. Leclercq (1997). Effect of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. Brit. Poult. Sci. 38:607-610.

AOAC (1990). Association of Official Analytical Chemists. Methods of Analysis 15th Edition, Assoc. Offic. Anal. Chem. Arlington, USA.

Atia, F. A.; P.E. Waibel; I.H. Hermes; C.W. Carlson and M.M. Walser (2000). Effect of dietary phosphorus, calcium, and phytase on performance of growing turkeys. Poult. Sci. 79:231-239.

BASF- informations (2000). Natuphos the key to phosphorus. Poult. International, 39: 11.

Broz, J.; P. Oldale; A. H. Perrin-Voltz; G. Rychen; J. Schulze and C. S Nunes (1994). Effect of supplemental phytase on performance and phosphorus utilization in broiler chickens fed low phosphorus diets without addition of inorganic phosphates. Br.Poult. Sci.35: 273-280.

Brune, H. and K. Gunther (1959). Die anvendung der Variabilitat des physiologischen indicatorssystems Wachstum und Ossifikation bei der Ratte zur biologischen Bestimmung von Calcium und Phosphorsalzen. Z. Tierphysiol., Tierernahahrg. U. Futtermittelkde.14: 76.

Calvert, C.C.; R.J. Besecker; M.P. Plumlee; T.R. Cline and D.M. Forsyth (1978). Apparent digestibility of phosphorus in barley and corn for growing swine. J.Anim. Sci. 47: 420-426.

Dale, N.M and L. Fuller (1980). Effect of diet composition on feed intake and growth of chicks under heat stress. II. Constant vs. cycling temperature. Poult. Sci. 59:1434-1441.

Denbow, D. M, V. Ravindran, E. T. Kornegay, Yi, Z. and R. M. Hulet (1995). Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poult. Sci.74: 1831-1842.

Duncan, D. B. (1955). Multiple Range and multiple F.Tests.Biometrics.11: 1-42.

El-Deeb, M. A.; H. Sharara and M. N. Makled (2000). Enhance calcium and phosphorus utilization by enzyme phytase supplemented to broiler diet contained rice bran. Egypt. Poult. Sci.J., 20: 545-566.

Gunther, K. D.; I. H. Hermes; S. E. Eyo and HJ. Abel (1982). Evaluation of the intermediary availability and the efficiency of utilization of feed phosphorus in growing chicken. 2- the effect of phosphorus amount in the feed. Z. Tierphysiol, Tierernahahrg. U. Futtermittelkde. 48: 260-266.

Hermes, I. H. (1982). Influence of nutritive growth stimulating substances on phosphorus turnover in chicken. Gesellschaft fur Ernahrungsphysiologie der Haustiere. 36. Tagung in Giessen von 24-26 Marz, 1982.

Hermes, I. H.; K. D. Gunther and HJ. Abel (1983). Evaluation of the intermediary availability and the efficiency of utilization of feed phosphorus in growing chicken. 3– The influence of the weight proportion between Ca and P in the feed. Z. Tierphysiol., Tierernahahrg. U. Futtermittelkde. 49:24-30.

Hermes, I. H.; B.M. Ahmed; M. H. Khalil; M.S. Salah and A.H. Al-Homidan (1999). Growth performance, nutrient utilization and carcass traits of growing Californian rabbits raised under different ambient temperatures. Egypt. J. of Rabbit Sci. 9:117-138.

Hussein, A. S. (1996). Effect of dietary energy and vitamin C on growth performance of broiler chicks raised in hot climates. Emirates J. of Agricult. Sci. 8:49-62.

Jongbloed, A.W. and P. A.Kemme (1990). Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. Neth.J.Agric.Sci.38: 567-575.

Kesbavarz, K. (1996). Phytase and phosphorus requirement of laying hens. Poult. International, 35:100.

Knuckles, B.E and A.A.Betschart (1987). Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. J.Food Sci. 52:719-721.

Kornegay, E.T.; D.M. Denbow. Z. Yi and V. Ravindran (1996). Response of broilers to graded levels of microbial phytase added to maize-soybean meal –based diets containing three levels of nonphytate phosphorus. Brit.J.Nutr.75: 839-852.

Mebta, R.K.; and B.K. Sbingari (1999). Feeding in heat stress. Poult. International, 38:68-77.

Nelson, T.S. (1967). The utilization of phytate P by poultry –A review. Poult. Sci. 46: 862-871.

Nelson, T.; S. Shieh; T. R. Wodzinski and J.H. Ware (1968). The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. Poult. Sci. 47: 1842-1848.

NRC (1994). National Research Council, Nutrient Requirements of Poultry. 9th rev . ed . National Academy Press, Washington, DC.

Ravindran, V.Y, E.T. Kornega, D. M Denbow, Z. Yi, and R.M. Hulled (1995). Response of turkey poults to tiered levels of Natuphos phytase added to soybean meal-based semi-purified diets containing three levels of nonphytate phosphorus. Poult. Sci. 74: 1820-1830.

Reddy, N. R, S.K.Sathe, and D.K.Salunkhe (1982). Phytase in legumes and cereals. Pages 1-91 in; Advances in Food Research, C.O.Chichester, E.M.Mrak, and G.F.Stewart, ed, Academic Press, New York, NY.

SAS (1996). SAS Procedure Guide. Version 6.12 Edition. SAS Institute INC., Cary, NC, USA.

Sauveur, B. (1978). Phosphorus requirements in finishing broilers aged 4 to 8 weeks. Arch. Geflugelkd. 42:229.

Saylor, W.W. (2000). Reduce phosphorus in diets to protect the environment. World Poult., 16:16-18.

Sebastian, S.; S. P. Touchburn; E. R. Chavez and P. C. Lague (1996). The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chicks fed corn-soybean diets. Poult. Sci. 75: 729-736.

Schoner, V. F. J; P. P.Hoppe; and G. Schwarz (1991). Comparative effects of microbial and inorganic P on performance and on retention of P, Ca and crude ash in broilers. J. Anim. Physiol. and Anim. Nutr. 66:248-255.

Simon, O.; H. Gruppen; H. Classen; W. Close; N. Khan; T. Acamovic, B. V. McCleary, A. Morgan and D. Cowan (1996). Enzymes in action, a feed mixes special. World Poult. Misset., 11: 62-71.

Simons, P. C.; M. Versteegh; H. A.J. Jongbloed; P. Kemme; K. D. Stump; M. G. Bos; E. Wolters; R. Beudeker and G. j. Veschoor (1990). Improvement phosphorus availability by microbial phytase in birds and pigs. Br.J.Nutr.64: 525-540.

Sohail, S. S, and D. A. Roland (1999). Influence of supplemental phytase on performance of broilers at four to six weeks of age. Poult.Sci.78: 550-555

Teeter, R.G. (1995). Optimizing production of heat stressed broilers. Proceeding of the International Conference on Animal Production in Hot Climates. Muscat. Sultanate of Oman, 8-10 January, 1995:129-137.

Tortuero, F. and M. V. Diez Tardon (1983). Possibilities in the use of low phosphorus concentrations for broiler diets during the finishing period. Adv. Aliment. Mejora Anim. 24:63.

Wilson, E. K.; F.W. Pierson; P.Y. Heser; R.L. Adams; and W.J. Stedelman (1980). The effect of high environmental temperatures on feed passage time and performance traits of White Pecking ducks. Poult. Sci. 59: 2322-2330.

Yi, Z.; E. T Kornegay; V. Ravindran and D. M. Denbow (1996). Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of P equivalency values for phytase. Poult. Sci.75: 240-249.

Yoshida, M. and H. Hoshii (1982). Re-evaluation of requirement of calcium and available phosphorus for starting meat-type chicks. Jpn. Poult. Sci. 19: 101

Yousef, M.K. (1985). Stress Physiology in Livestock, Volume III-Poultry, (Feeding poultry in hot and cold climates, PP: 123-136). CRC Press, Inc. Boca Raton, 33431 Florida, USA.

# تأثير إضافة إنزيم الفيتيز على الأداء الإنتاجي وتكلس العظم للـدجاج اللاحـم تحـت ظروف الإجهاد الحراري

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أجرى هذا البحث بهدف دراسة تأثير إضافة إنزيم الفيتيز على الأداء الإنتاجي ودرجة تكلس العظم للدجاج اللاحم تحت ظروف الإجهاد الحراري. أجريت تجربة عامليه ٢ ×٢ ×٢ حيث استخدم مستويين من درجات الحرارة (٢٠ ، ٣٠ م°) ، مستويين من الفسفور المستفاد (٠,٣٤ ، ٠,٤٥ % ) ، مستويين من إنزيم الفيتيز (صفر ، ٥٠٠ وحدة/كجم غذاء) وكانت نسبة الكالسيوم الكلى : للفوسفور المستفاد ثابتة ٢,٢٢ :١ .أستخدم في الدراسة ٢٤٠ كتكوت لاحم عمر يوم من سلالة هيبرو غير المجنسة واستمرت لمدة ٥ أسابيع. تم تسكين الكتاكيت في حجرتين مستقلتين بنظام التربية الأرضية في عدد ٨ وحدات مستقلة بكثافة ١٥ طائر/ م$^2$ داخل عنبر مغلق. تم دراسة درجة تكلس العظـم (عظمـة الساق اليمنى) في نهاية التجربة على عدد ٦ طيور (٣ذكور+ ٣ إناث) من كل معاملة ومكررة.

أوضحت النتائج التأثير المعنوي السالب لدرجات الحرارة المرتفعة على كل من وزن الجسـم ، كميـات الغـذاء المستهلك وكفاءة تحويل الغذاء (غذاء/ نمو) في جميع الأعمار المختبرة خاصة من عمر الفقس حتى عمر ٣٥ يـوم وكذلك درجة تكلس عظمة الساق عند عمر ٣٥ يوم مقارنة بتلك المرباة تحت نظام الحرارة المقارنة (٢٠ م°) .في معظم الأحيان أدى إضافة ٠,٣٤ % فوسفور مستفاد إلى زيادة معنوية في كل من وزن الجسـم ، كميـات الغـذاء المستهلك وكفاءة تحويل الغذاء مقارنة بالمستوى المرتفع (٠,٤٥ %)، بينما درجة تكلس عظمة الساق كـان أعلـى معنويا عند استعمال المستوى المرتفع من الفسفور المستفاد (٠,٤٥ %) مقارنة بالمستوى المنخفض (٠,٣٤ %).أدى إضافة إنزيم الفيتيز بمستوى ٥٠٠ وحدة/كجم غذاء إلى تحسن في وزن الجسم ومعدل الزيادة اليومية في الوزن وكذلك كفاءة تحويل الغذاء ، وكذلك درجة تكلس عظمة الساق ، بينما لم يتأثر معدل استهلاك الغذاء مقارنة بالعليقة الخالية من إنزيم الفيتيز .كان تأثير التفاعل بين العوامل الثلاث التي تم دراستها (درجة الحرارة ×مستوى الفسفور المسـتفاد ×إضافة إنزيم الفيتيز) معنويا ($p<0.05$) على كل من وزن الجسم عند عمر ٣٥ يوم ، معدل الزيادة اليومية فـي الوزن وكفاءة تحويل الغذاء (من عمر الفقس حتى عمر ٣٥ يوم) ، ودرجة تكلس عظمة الساق (عند عمر ٣٥ يوم). بينما لم يظهر هذا التداخل أي تأثير معنوي على معدل استهلاك الغذاء (من عمر الفقس حتى عمر ٣٥ يوم) . مما سبق يتضح انه عند تغذية الدجاج اللاحم على علائق تعتمد على الذرة الصفراء وكسب فول الصويا مع استعمال ٠,٣٤ % فوسفور مستفاد ، وكانت نسبة الكالسيوم الكلى : للفوسفور المستفاد ثابتة ٢,٢٢ :١ وبدون استعمال إنـزيم الفيتيـز بمستوى ٥٠٠ وحدة/كجم غذاء تعتبر كافية لتغطية الاحتياجات الغذائية للدجاج اللاحم من عمر يوم وحتى ٥ أسـابيع (وزن الجسم ، معدل الزيادة في النمو ، معدل استهلاك الغذاء وكفاءة تحويل الغذاء) بينما تعتبر غير كافية لزيادة درجة تكلس عظم الساق بدرجة عالية.

من نتائج هذه التجربة ينصح تحت ظروف الإجهاد الحراري في حدود (٣٠ م° ) باستعمال إنزيم الفيتيز بمستوى ٥٠٠ وحدة/كجم غذاء مع إضافة المستوى المنخفض من الفسفور المستفاد (٠,٣٤ % ) على أن تكون نسبة الكالسيوم الكلى : للفوسفور المستفاد ثابتة ٢,٢٢ :١ حيث يؤدى إلي تحسن عام في الأداء الإنتاجي للدجاج اللاحم مع زيادة في درجة تكلس العظم بدرجة مرضية من عمر يوم حتى ٥ أسابيع.