

ROLE OF PHYTASE SUPPLEMENTATION INTO MUSCOVY DUCKG DIET IN THERMO - AND OSMOREGULATION DURING SUMMER SEASON

By

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ABSTRACT: *A total of 126 Muscovy ducklings one day old were used in the present study to assess the effect of phytase supplementation on HSP70 gene expression, thermal reaction, plasma osmotic pressure, hematological parameters, some plasma parameters. liver and kidney functions during summer conditions. Ducklings were divided into six equal groups. Three diets were formulated to contain 0.25, 0.34 and 0.45% NPP for feeding from 1 d to 3 weeks of age, and 0.21, 0.30 and 0.40 % NPP for feeding from 3 to 11 weeks of age. respectively. Phytase enzyme was added to all diets at two levels (0 and 750 U/Kg).*

Results showed that HSP70 and respiration rate increased significantly with increasing dietary NPP level. Enzyme addition into diets caused a significantly increase in HSP70 compared with other treatments while, body temperature did not differ significantly by dietary NPP level without or with enzyme addition. With respect to liver (as measured by AST and ALT) or kidney functions (as measured by uric acid and creatinine levels), ducks fed diet contained lower NPP recorded higher levels of these plasma components than the other groups. While, addition of phytase to ducks diet had no deleterious effect on liver or kidney function. Plasma osmotic pressure significantly decreased with increasing NPP level and phytase supplementation.

Neither phosphorus levels nor phytase inclusion had significant effect on WBC, RBC and PCV. While, hemoglobin concentration increased significantly with increasing dietary NPP level. Enzyme addition into diets caused a significant increase in hemoglobin concentration (Hb) compared with other treatments.

Present data indicates that phytase supplementation had some beneficial effects on some physiological, hematological parameters and plasma osmotic pressure in Muscovy ducks during summer conditions.

ducks. HSP70, body temperature, respiration rate, plasma osmotic pressure, hematological parameters, summer conditions

INTRODUCTION

The hydrolysis and absorption of phytate phosphorus (P) by monogastric animals are complex process that are influenced by many factors. Dietary ingredients and feed processing seem to be the most important factors related to the diet, while age and type of birds could also affect phytate (P) utilization (Reddy *et al.*, 1982; Sebastian *et al.*, 1998 and Attia, *et al.*, 2003). This possesses a problem to nonruminant animals because they do not produce sufficient amounts of intrinsic phytase necessary to hydrolyze the phytic acid complex.

Also, phytate can form various salts with the important minerals such as Ca, Mg and Cu. (Sebastian *et al.*, 1998).

On the other hand, some studies on chicks indicated that P deficiency impaired their immune response (Aslam, 1995).

In general, prokaryotes and eukaryotes respond to stressors at a cellular and molecular level with a universal expression of heat shock proteins (HSP) (Craig, 1985; Lindquist, 1986; Morimoto *et al.*, 1990). Heat shock proteins are the most broadly distributed classes of protein known and are among the most highly conserved in nature. The most prominent HSP of mammalian and avian cells have molecular masses of approximately 90 kDa (HSP 90), 70 kDa (HSP 70) and 27 kDa (HSP 27) (Schlesinger, 1986; Morimoto *et al.*, 1990 and Wang, 1992). Heat shock proteins (HSPs) are believed to be involved in cellular protection against many 'adverse environmental or physiological stimuli (Lindquist and Graig, 1988 and Maloyan *et al.*, 1999). Also, Edens *et al.*, (1992) studied the effect of dietary phosphorus on translation of HSP 90, HSP 70 and HSP 23 in heat stressed broiler chickens. Broilers that were fed a Pi-deficient diet had significantly lower expression of HSP 70, HSP 90 and HSP 23 proteins when they were challenged by acute heat stress for 1 h., who postulated that acute heat stress susceptibility of Pi-deficient chickens might be due to their inability to initiate a normal HSP response to acute heat exposure.

MATERIAL AND METHODS

The experiment was carried out in the Research Station of Waterfowls at ElSerw, Domiat Governorate, which belongs to the Animal Production Research Institute, Ministry of Agriculture, Egypt. The experiment was carried out during summer months (July, August and September). A number of 126 one-day old Muscovy ducklings were used. They were randomly and equally distributed into 6-treatment groups (21 ducklings each). Gas heaters were used to keep the required temperature for the brooding period while light was provided 23 hr daily throughout the experimental period. Feed and water were allowed for *ad libitum* consumption.

During the experimental period, the average minimum and maximum indoor temperatures and relative humidity were 28 , 39°C and 65%, respectively.

Tables 1 and 2 show the formulation and nutrient composition of the starter and grower diets, respectively. Phytase enzyme (Ronozyme 2500) was added (750 FTU/Kg) to diets 100, 75 and 50% and these diets were fed with or without phytase supplementation. Thus a number of 6 diets were formulated.

Heat shock proteins 70 (HSP70) gene expression, thermal reaction, plasma osmotic pressure, hematological parameters, some plasma parameters, liver and kidney functions were determined as affected by dietary non-phytate phosphorus (NPP) level and phytase supplementation during summer season.

A starter (22% CP and 2900 Kcal ME/Kg) and a grower (17% CP and 3000 Kcal ME/Kg) basal diets (diet 100%) were formulated with no inorganic phosphorus supplementation. The starting period lasted from 1 to 21 days of age while, the growing period lasted from 3 to 11 weeks of age. Nutrients content of such basal diets were adequate to cover the recommended duck requirements (NRC, 1994) except that of P. The calculated total P (tP) and NPP contents were 0.69 and 0.45% for the starter diet and 0.61 and 0.40% for the grower diet, respectively. Commercial dicalcium phosphate (18.7% P and 22% Ca) was added to formulate diets of treatments 75 and 50% containing 0.58 and 0.49% tP and 0.34 and 0.25 % NPP, respectively in starter period, and 0.51 and 0.42% tP and 0.30 and 0.21% NPP, respectively in grower period. Limestone and vegetable oil were used to adjust dietary Ca and calories content, respectively. Vitamin and mineral mixture was added in enough quantities to cover the duck requirements (NRC 1994).

Physiological responses measurement

Five birds were randomly taken for measuring rectal temperature (as a measure of body temperature) and respiration rate were measured at 2.00 a.m. during the end of experimental period. Rectal temperature was obtained gently by thermometer inserted into the cloacae and respiration rate by counting the body wall movement for one minute using stop watch and a counter. Blood samples (about 3ml heparinized blood / bird) were collected from the brachial vein of 5 birds each group. Blood pH was determined by using digital electric pH meter (JENCO model No. 608 U.S.A) immediately after blood samples collection.

Blood samples were divided into two portions. The first portion was immediately centrifuged at 3000 rpm for 15 minutes and plasma was then separated and stored at -20°C for assaying of calcium (mg/100 ml), Pi (mg/100 ml) and cholesterol (mg/100 ml) were determined in plasma according to Wootton (1982), Gomorri, (1942) and Richmond (1973), respectively.

AST (Aspartate-aminotransferase) and ALT (Alanine-aminotransferase) activities were determined using the method described by White (1970), creatinine and uric acid were determined using the method described by Husdan, (1968) and Bogin and Keller (1987), respectively. Plasma sodium (Na) and potassium (K) were determined using the method described by Dean (1960).

Plasma osmotic pressure was measured by a vapor pressure osmometer (Wescor 5500, USA).

The second blood portion was taken to determine haemoglobin (g/dl) Packed-cell volume (%) red blood cells ($\times 10^6/\text{mm}^3$) and white blood cells ($\times 10^3 \text{ mm}^3$).

Haematocrit value was determined and expressed as a percentage of packed cell volume (PCV %) according to Hunsaker (1969), haemoglobin concentration was determined in fresh blood samples using haemoglobinometer as the method described by Pilaski (1972). Red blood cell's (RBC's) and white blood cell's (WBC's) were counted in fresh blood sample as the method described by Hawkey and Dennett (1989) using haemocytometer and counted at 400 X objective of a phase contrast microscope.

MRNA heat shock protein 70

Samples of brain tissue from three ducks were excised immediately and rapidly stored in open dorff tubes and kept in liquid nitrogen until

analysis. Total cellular RNA was extracted by using kit (QIAGEN, 1999) and it was spectrophotometrically determined at 260nm.

Reverse Transcription Polymerase Cycle Reaction (RT-PCR).

One mg of total RNA was reverse-transcribed using kit (One-Step RT-PCR ABgene UK) in a total reaction volume of 50.1.1.1 of reverse transcription product (cDNA) was amplified using aml-taq DNA polymerase (ABgene UK) and 0.5.1 and 1.1 of HSP 70 from forward and reverse primers (Saiki, *et al.* 1988). The RT-PCR cycle consisted of annealing at 48 °C for 45 minutes. Subsequently, samples were incubated at 94 °C for 2 minutes, 59 °C for 1 minute and 68 °C for 1.5 minutes for 45 cycles. Final extenuation was carried out at 68 °C for 10 minutes.

Quantitation of DNA by ethidium bromide fluorescence

cDNA concentration was determined by diluting the DNA (1:5) (2.1 of DNA+8.dd. H₂O). DNA samples were loaded in 0.7% agarose gel and run against 10.1 of a suitable DNA size marker (DNA digested with Hind III and .X 174 DNA digested and 310 bp (base pair). DNA was visualized using a 302 nMUV transilluminator to show the stained DNA bands by ethidium bromide stain (Sambrook *et al.*, 1989). Gels were photographed using a Polaroid camera. The Gal analyzer software (GS 365 data system) was used to determine HSP 70 cDNA.

Statistical analysis:

Data were analyzed by the least squares analysis of variance using the General Linear Models procedure of the statistical analysis model (SAS, 2001). The statistical model was as follows:

$$Y_{ijk} = \mu + T_i + A_j + TA_{ij} + E_{ijk}$$

Where: Y_{ijk} . = Observation of the ij^{th} duck: μ = Overall mean, common element to all observations: T_i = Effect of dietary NPP level treatment ($i = 1..2. 3$); A_j = Effect of phytase supplementation ($j = 1,2$); TA_{ij} = Interaction effect between i^{th} dietary NPP level treatment and j^{th} phytase supplementation: and E_{ijk} = Random error component assumed to be normally distributed. Data estimated in percentage were transformed with the aresine square-root procedure to normalize variance before analysis and were retransformed again to the original scale before presentation. The differences among means were tested using Duncan's New Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Gene expression of heat shock protein 70 (HSP70)

Results showed that, gene expression of HSP70 was affected by dietary NPP level and also enzyme addition into diets (Fig 1 and 2) whereas; it increased with increasing dietary NPP level and enzyme addition into diets followed by the other treatments except birds fed diets of 50% NPP without enzyme addition which recorded the lowest gene expression of HSP70 compared to other treatments during summer season. This result is in agree with Mahmoud *et al.* (2004) who indicated that Pi deficiency in chickens diet inhibited transcription of HSP in certain organs when they were exposed to heat stress. On the other hand, Edens *et al.*, (1992) reported lower translational process of HSP due to phosphorus availability in the diet which was associated with both HSP mRNA type and organ. Finally, dietary Pi had a positive effect on HSP protein expression that was manifested during translation of HSP mRNAs. Thus, Pi deficiency may affect major cellular biochemical pathways that control protein expression (Mahmoud *et al.*, 2004).

Thermal Reaction

Data in Table (3) showed that respiration rate was affected by dietary NPP level. Whereas, it was increased significantly with increasing dietary NPP level and also, enzyme addition into diets causing a significantly higher respiration rate compared to other treatments.

Also data in Table (3) showed effect of interaction between dietary NPP level and phytase supplementation during summer season on respiration rate whereas enzyme addition with the highest NPP level into diets caused a significant increase in respiration rate compared to other treatments. This result agrees with Namra. (2006).

Data in Table (3) show that body temperature did not differ significantly by dietary NPP level without or with enzyme addition. Also, body temperature no affected by the interaction between dietary NPP level and phytase supplementation during summer season. Sturkie (1986) reported that body temperature of bird depend on bird size, environmental temperature, age and sex. The rise in body temperature in response to high environmental temperature in the present study was also reported previously by EI-Gendy and Washburn (1995) who indicated that rectal temperature is a good indicator to both of heat stress and acclimation. In general, body temperature and respiration rate improved in birds supplemented with phytase under summer condition. The same beneficial effect of phytase

supplementation on thermoregulation under summer condition could be attributed to dissipating more heat (Namra, 2006). This study suggests that the increase in respiration rate of birds during high environmental temperature is due to evaporating heat-dissipating mechanism that maintains their normal body temperature.

Blood pH Values

Result presented in Table (3) indicated that average values of blood pH were slightly significantly decreased with decreasing NPP level, without phytase supplementation to ducks diets. This may be due to the decrease of respiration rate in these groups.

Elevation of blood pH which was concomitant with increase in RR in the present study was interpreted by Balnave and Gorman (1993). They stated that carbon dioxide is an end product of oxidative metabolism in tissues and is converted to carbonic acid through the action of the enzyme carbonic anhydrase. Carbonic acid a weak acid is a source of bicarbonate ion. However, it is as a component of the bicarbonate/ carbonic acid buffer system that the bicarbonate ion exerts its primary function. This is the most important buffering system plasma and it interacts with the respiratory and renal systems in regulating the acid-base status of the plasma. The renal system regulates the loss of bicarbonate while carbon dioxide loss is mediated by the lungs. Under heat stress conditions birds regulate heat loss through the evaporation of water from their lungs. While both panting and non-panting heat stressed birds exhibit reduced levels of plasma carbon dioxide and bicarbonate, the induction of panting result additional respiratory alkalosis Teeter *et al.* (1985). This respiratory alkalosis causes disruption in blood flow pattern, body water distribution and mineral and ionic balance (Smith and Teeter, 1993).

The influence of high ambient temperature on blood pH is conflicting. For example, Brees *et al.* (1989) observed that pH increased in a curvilinear fashion when chicken was exposed to increasing ambient temperature changes. While, Siegel *et al.* (1974) found no difference in blood pH for broiler reared under continuous 35°C vs. thermoneutral conditions. Reasons for these discrepancies are not clear but may include degree of thermal stress type of stress (Acute vs. chronic), blood collection site, sampling time relative to the respiratory state of birds or acclimatization of birds to continuous exposure to heat stress.

Data in Table (3) showed that, the main effect of NPP level and phytase supplementation on blood pH was when birds were fed 100% NPP without phytase supplementation, as it recorded the significantly highest blood pH

values compared to other treatments.

Hematological Parameters

Data of hematological parameters are presented in (Table, 4) indicated that neither phosphorus levels nor phytase inclusion had significant effect on WBC, RBC, and PCV. Also, the interaction between dietary NPP level and phytase supplementation during summer season did not affect these parameters.

However, a slight improvement could be observed in these parameters with increasing phytase and NPP level. This is in contrast to the data obtained in chicks by Aslam (1995) who observed increased chick antibody titers to sheep red blood cells with increasing concentrations of dietary nonphytate P. This enhancement of immunity may occur corresponding to phytase supplementation as a result of improving feed consumption, absorption and utilization of nutrients. These results are in agreement with Mervat *et al* (2001) who found that values of WBC, RBC and PCV were not affected by dietary NPP and phytase treatments.

Data in Table (4) showed that, hemoglobin concentration was affected by dietary NPP level. Whereas, it increased significantly with increasing dietary NPP level. Enzyme addition into diets caused a significant increase in hemoglobin concentration (Hb) compared to other treatments. The increase in Hb value may be connected to the effect of phytase supplementation on iron absorption, mobilization and distribution through the body (Erdman, 1979).

Enzyme addition with the highest NPP level into diets caused a significantly higher hemoglobin concentration compared to other treatments

Values of WBC, RBC and PCV were not affected by interaction between dietary NPP and phytase treatments.

Liver enzymes

With respect to AST and ALT Table (5) showed that ducks fed diet contained lower NPP or without phytase recorded highest levels of AST and ALT compared to other groups. The similitude of enzyme activity in supplemented phytase groups is exhibit healthy, non pathological or non toxic effects of tested biological additives on liver function.

Changes in blood transaminases level may depend on the rate of protein metabolism which may be a function of bird's age rather than any other factor. It is well known that by the simple process of transamination, an amino radical is transferred to alfa- keto acid while the keto oxygen is

transferred to the donor of the amino radical who is promoted by transaminases (Guyton, 1981).

Data in Table (5) showed that, the main effect of NPP level and phytase supplementation on liver function. Birds fed 50% NPP without phytase supplementation recorded significantly highest levels AST and ALT compared to other treatments.

Kidney function

Uric acid is the major nitrogenous end product of nitrogen metabolism in birds. Since, it is the excretory vehicle for four fifths of the metabolized nitrogen. Both uric acid and creatinine levels in plasma as protein metabolites followed the same manner as total plasma protein. These levels increased or decreased in response to protein metabolism.

Data in Table (5) showed that there were significant differences in plasma creatinine and uric acid levels among the different dietary treatments; Ducks fed phytase had significantly decreased averages of plasma creatinine and uric acid levels. Suggesting that kidneys of birds were affected by enzyme supplements applied and increasing NPP level. Whereas, from the previous studies it can be stated that phytase significantly affects crude protein digestibility at the diets. Such improve in crude protein digestibility could be explained by releasing protein and / or amino acids from phytate-protein complexes. Present results indicate that protein metabolism had significant effect on both parameters. These agree with the results obtained by Geraert *et al.* (1996) in broilers.

Also data in Table (5) shows effect of interaction between dietary NPP level and phytase supplementation during summer season on plasma creatinine and uric acid as enzyme addition with the highest NPP level into diets caused a significant decrease in plasma creatinine and uric acid compared to other treatments.

From data obtained on the previous blood parameters it could be concluded that addition of phytase to ducks diet had no adverse effects on liver (as measured by AST and ALT) nor kidney function (as measured by uric acid and creatinine levels). Generally, these findings are in agreement with those of Pescatore *et al.* (1990) who reported that the numeric variations in blood parameters could be interpreted due to many factors such as genetics, age, sex, physiological state, rearing conditions feeding as well as pathological factors.

Plasma Ca and P

Levels of plasma Ca and P at 11 wks of age (Table. 6) showed that there were significant effect of phosphorus level on plasma Ca and P. It was noticed that groups fed 50 and 75% available P recorded insignificantly higher Ca and significantly lower P than other P levels which recorded similar levels of plasma Ca values, in spite of increased plasma P with increasing dietary available P. The lower plasma P level in birds fed diet containing 50 and 75% available P may be due to increased plasma Ca in these groups. Lima *et al.* (1997) reported that blood P increased with feeding high P level diet. This result agrees with Perney, *et al.* (1993) who found that plasma P increased by increasing dietary available P.

Phytase addition only had no effect on plasma Ca. This agrees with Scheideler and Ferket, (2000) who reported that serum P and Ca were not affected when broiler chicks were fed 0.42 and 0.30% av.P and 0.30% av.P with phytase supplementation.

In view of the results obtained at 11 wks of age, it could be concluded that the effect of dietary P level on plasma P was significant ($P < 0.05$). Plasma P was increased with increasing dietary P level while plasma Ca was insignificantly decreased. Decrease a plasma Ca could be explained by high blood phosphate levels depressing the formation of vitamin D in the kidneys, which reduce blood calcium. The results reported herein agree with Lima *et al.*, (1997) who reported that plasma P increased with increasing dietary P level but plasma Ca was decreased.

The effect of phytase on plasma P was significant ($P < 0.05$). Phytase supplementation insignificantly decreased plasma Ca and increased plasma P. Moreover, addition of phytase releases a large amount of P from phytate-bound P and leads to high blood phosphate levels which reduce blood Ca as the adverse relationship mentioned above. This agrees with Sebastian *et al.* (1996) who found that phytase addition in broiler diets reduced plasma Ca. Similar results were reported by Lou-Hong Zing *et al.*. (1997) who reported that blood P was increased by phytase supplementation to broiler diets. In all treatments, it was noticed that phytase addition insignificantly decreased plasma Ca. This effect was clear in birds fed diets containing low-P level (50 and 70% available P). In contrast, addition of phytase increased plasma P level.

It could be concluded that increasing dietary av.P level increases plasma P and insignificantly decrease plasma Ca. Regardless of phosphorus level, phytase addition had no significant effect on plasma Ca but it increased plasma P.

Plasma cholesterol

It is well known that serum or plasma cholesterol of birds is strongly affected by heredity, nutrition, age, sex and environmental conditions (Sturkie, 1986). The results of plasma cholesterol at 11 wks of age (Table. 6) showed that ducks fed diet contained lower NPP or without phytase recorded higher levels of plasma cholesterol compared to other groups.

This decrease observed in blood cholesterol levels may be due to occur in the rate of cholesterol absorption through the intestinal villi that may be reflected as decreased level in the blood or this low level of plasma total cholesterol may be also due to phytase acting on the enzyme 7- α hydroxylase which controls cholesterol catabolism (Wahba, 1969).

This decrease may be significantly logic since; the cholesterol was oxygenated in the liver and results in the formation of bile acids which occur as anions (Life salts). Bile salt excreted into the intestine for oil digestion (Stroev, 1989). In addition, Wahba (1969) who reported that, bile salts are important end products in the metabolism of cholesterol.

Osmoregulation:

Phosphorus helps the kidney function and acts as a buffer for acid base balance in the body (Underwood and Suttle, 1999). At 11 wks of age the main effects of dietary av.P level or phytase supplementation were not significant on plasma Na and K (Table, 7). Birds reared under summer condition and fed low NPP level or without phytase supplementation showed insignificantly lower plasma Na and K compared to other treatments (Table. 7). Plasma osmotic pressure at 11 wks of age showed different responses to dietary av.P level under summer condition as indicated by its significant interaction (Table. 7), where under summer condition increasing NPP level significantly decreased plasma osmotic pressure compared to those fed low NPP level.

Phytase supplementation also significantly reduced plasma osmotic pressure under summer condition (Table. 7). These results are very close to those of Yahav *et al.* (1997) who reported that plasma osmolality in broilers hardly changed during the temperature cycle of 10-30 °C. However, during the 35 °C phase of the 15-35 °C cycle plasma osmolality was reduced significantly compared with that at 25 °C. Also, they added that the increase in concentration of plasma protein could be important in this regulatory response, which also resulted in a decrease in osmolality.

In summary birds at 11 wks of age fed high NPP level with phytase supplementation showed a slight increase in both plasma Na and k. The

most prominent result of birds aged 11 wks was the significant reduction in plasma osmotic pressure in response to phytase supplementation.

The lack of significance of the main effect of dietary av.P level or phytase supplementation under summer condition on plasma Na and K at 11 weeks of age Table, 2) agreed with, Arad *et al.* (1983) and Arad and Skadhauge (1984) who suggested that high environmental condition with water available did not result in any changes in plasma Na, K and osmolality. They added that dehydration was responsible for the changes in these electrolytes. In addition, no expected differences in plasma volume were indicated due to the insignificant change in PCV% (Table, 4). These may indicate that birds of previous groups were in the same hydration state and the dehydration was not responsible for the reduction of plasma electrolytes. This confirms that enzyme addition is responsible for the slight increase of plasma Na and K that may cause beneficial effect on intracellular osmolality.

This substantial shift in K concentration of birds fed phytase supplementation may cause serious disturbance to the cells due to its effect on intracellular osmolality (Etches *et al.* 1995).

It could be concluded that, phytase supplementation showed beneficial effects on thermo- and osmoregulation as represented by insignificantly RR and blood hemoglobin, uric acid, creatinine and Ca^{++} P, lowering body temperature and plasma osmotic pressure under summer condition.

Table (1): Formulation and nutrient composition of starter diets.

Item	Diet 0.45 NPP (100%)	Diet 0.34 NPP (75%)	Diet 0.25 NPP (50%)
Ingredients (%)			
Yellow corn	54.25	54.51	54.65
Soybean meal (44%)	40.35	40.40	40.35
Cotton seed oil	2.06	1.95	2.00
Lime stone	1.05	1.45	1.81
Di calcium phosphate	1.60	1.00	0.50
Premix*	0.30	0.30	0.30
Salt	0.25	0.25	0.25
DL- Methionine	0.14	0.14	0.14
Total	100	100	100
Calculated composition (%)			
Crud protein	22.011	22.05	22.04
ME (Kcal /Kg)	2901.50	2901.75	2909
Crud Fiber	4.19	4.20	4.20
Crud Fat	4.72	4.62	4.68
Calcium	0.903	0.902	0.92
Avail. Ph	0.45	0.34	0.25
Total Ph	0.69	0.58	0.49
Lysin	1.32	1.32	1.32
Methionine	0.50	0.50	0.50
Methionine + Cystin	0.86	0.86	0.86
Sodium	0.11	0.11	0.11

*Contents per 3 kg premix: Vit. A 10 M. I. U., Vit. D3 1 M. I. U., : Vit. E 10gm, : Vit. K3 1g, : Vit. B1 1g, : Vit. B2 4g, N. acid 10g, P. acid 10g, Vit. B6 105g, : Vit. B12 10mg, F. acid 1g, Biotin 50mg, Choline 500g, Zinc 45g, Copper 3g, Iodine 0.3g, Iron 30g, Selenium 0.1g, Manganese 40g and Carrier CaCo2 to 3000g.**According to NRC (1994)

Table (2): Formulation and nutrient composition of grower diets.

Item	Diet 0.40 NPP (100%)	Diet 0.30 NPP (75%)	Diet 0.21 NPP (50%)
Ingredients (%)			
Yellow corn	69.13	69.46	69.43
Soybean meal (44%)	26.50	26.50	26.50
Cotton seed oil	1.05	0.92	1.05
Lime stone	1.25	1.60	2.0
Di calcium phosphate	1.45	0.90	0.40
Premix*	0.30	0.30	0.30
Salt	0.25	0.25	0.25
DL- Methionine	0.07	0.07	0.07
Total	100	100	100
Calculated composition (%)			
Crud protein	17.023	17.048	17.04
ME (Kcal /Kg)	3000.706	3000.451	3010
Crud Fiber	3.523	3.531	3.53
Crud Fat	4.07	3.956	4.08
Calcium	0.902	0.90	0.92
Avail. Ph	0.40	0.30	0.21
Total Ph	0.61	0.51	0.42
Lysin	0.95	0.95	0.95
Methionine	0.37	0.37	0.37
Methionine + Cystin	0.66	0.66	0.66
Sodium	0.11	0.11	0.11

*Contents per 3 kg premix: Vit. A 10 M. I. U., Vit. D3 1 M. I. U., : Vit. E 10gm, : Vit. K3 1g, : Vit. B1 1g, : Vit. B2 4g, N. acid 10g, P. acid 10g, Vit. B6 105g, : Vit. B12 10mg, F. acid 1g, Biotin 50mg, Choline 500g, Zinc 45g, Copper 3g, Iodine 0.3g, Iron 30g, Selenium 0.1g, Manganese 40g and Carrier CaCo2 to 3000g.**According to NRC (1994)

Table (3): Thermal reaction and blood pH as affected by dietary NPP level and phytase supplementation during summer season.

Item	Phytase level	NPP level			SE	Over all mean
		100%	75%	50%		
Body temperature °C	0	40.53 ^a	40.51 ^a	40.53 ^a	±0.09	40.52 ^a ±0.05
	750	40.51 ^a	40.47 ^a	40.45 ^a		40.47 ^a ±0.05
	Over all mean	40.52 ^a	40.49 ^a	40.49 ^a	±0.06	
Respiration rate (breath / min)	0	51.20 ^b ^c	50.40 ^c	50.80 ^b ^c	±0.73	50.80 ^b ±0.47
	750	56.80 ^a	52.80 ^b	52.40 ^b ^c		54.00 ^a ±0.47
	Over all mean	54.00 ^a	51.60 ^b	51.60 ^b	±0.58	
Blood pH	0	7.77 ^a	7.68 ^{ab}	7.56 ^b	±0.05	7.26 ^b ±0.03
	750	7.21 ^c	7.26 ^c	7.31 ^c		7.67 ^a ±0.03
	Over all mean	7.49 ^a	7.47 ^a	7.43 ^a	±0.04	

^{a,b,c} Means with different superscripts in the same row within item differ significantly (P<0.05).

Table (4): Hematological parameters as affected by dietary NPP level and phytase supplementation during summer season.

Item	Phytase level	NPP level			SE	Over all mean
		100%	75%	50%		
RBC's x10 ⁶ /ml	0	2.68 ^a	2.68 ^a	2.65 ^a	±0.04	2.67 ^a ±0.02
	750	2.65 ^a	2.64 ^a	2.66 ^a		2.65 ^a ±0.02
	Over all mean	2.67 ^a	2.66 ^a	2.66 ^a	±0.03	
WBC's x10 ³ /ml	0	28.31 ^a	27.97 ^a	27.89 ^a	±0.66	28.05 ^a ±0.36
	750	28.04 ^a	27.87 ^a	28.24 ^a		28.05 ^a ±0.36
	Over all mean	28.17 ^a	27.92 ^a	28.06 ^a	±0.45	
PCV%	0	35.19 ^a	35.13 ^a	35.15 ^a	±0.36	35.16 ^a ±0.20
	750	35.24 ^a	35.24 ^a	35.17 ^a		35.21 ^a ±0.20
	Over all mean	35.22 ^a	35.18 ^a	35.16 ^a	±0.34	
Hb g/dl	0	12.03 ^b	12.27 ^b	11.94 ^b	±0.05	12.08 ^b ±0.28
	750	14.90 ^a	12.21 ^b	11.80 ^b		12.97 ^a ±0.28
	Over all mean	13.46 ^a	12.24 ^b	11.87 ^b	±0.35	

^{a,b,c} Means with different superscripts in the same row within item differ significantly (P<0.05)

Table (5): Liver and kidney function as affected by dietary NPP level and phytase supplementation during summer season.

Item	Phytase level	NPP level			SE	Over all mean
		100%	75%	50%		
Uric acid mg/100ml	0	5.08 ^b	4.86 ^b ^c	5.76 ^a	±0.14	5.23 ^a ±0.08
	750	4.06 ^d	4.52 ^c	5.07 ^b		4.55 ^b ±0.08
	Over all mean	4.57 ^b	4.69 ^b	5.42 ^a	±0.11	
Creatinine mg/100ml	0	15.45 ^b	15.54 ^b	16.14 ^a	±0.18	15.71 ^a ±0.11
	750	13.86 ^c	14.33 ^c	15.56 ^b		14.58 ^b ±0.11
	Over all mean	14.66 ^b	14.03 ^b	15.85 ^a	±0.14	
AST IU/l	0	126.77 ^b	128.90 ^{ab}	131.03 ^a	±0.83	128.90 ^a ±0.48
	750	117.07 ^d	121.49 ^c	121.92 ^c		120.16 ^b ±0.48
	Over all mean	121.92 ^b	125.20 ^a	126.47 ^a	±0.59	
ALT IU/l	0	40.69 ^a	40.85 ^a	41.39 ^a	±0.38	40.98 ^a ±0.23
	750	38.54 ^b	38.75 ^b	40.83 ^a		39.37 ^b ±0.23
	Over all mean	39.61 ^b	39.80 ^b	41.11 ^a	±0.28	

^{a,b,c} Means with different superscripts in the same row within item differ significantly (P<0.05).

Table (6): Plasma cholesterol, calcium and phosphorus levels as affected by dietary NPP level and phytase supplementation during summer season.

Item	Phytase level	NPP level			SE	Over all mean
		100%	75%	50%		
Ca mg/dl	0	9.76 ^a	9.84 ^a	9.72 ^a	±0.33	9.77 ^a ±0.18
	750	10.07 ^a	10.10 ^a	10.34 ^a		10.17 ^a ±0.18
	Over all mean	9.91 ^a	9.92 ^a	10.03 ^a	±0.23	
Ph mg/dl	0	4.99 ^b	4.27 ^b	4.13 ^c	±0.12	4.62 ^b ±0.11
	750	6.27 ^a	5.12 ^b	5.11 ^b		5.50 ^a ±0.11
	Over all mean	5.63 ^a	4.93 ^b	4.62 ^b	±0.13	
cholesterol mg/dl	0	159.07 ^c	165.19 ^a	162.69 ^{ab}	±1.92	162.32 ^a ±0.66
	750	152.01 ^d	157.13 ^c	160.33 ^{bc}		156.46 ^b ±0.66
	Over all mean	155.54 ^b	161.16 ^a	161.46 ^a	±0.81	

^{a,b,c} Means with different superscripts in the same row within item differ significantly (P<0.05)

Table (7): Plasma sodium, potassium levels and osmotic pressure as affected by dietary NPP level and phytase supplementation during summer season.

Item	Phytase level	NPP level			SE	Over all mean
		100%	75%	50%		
Na mmol/l	0	162.45 ^a	162.32 ^a	162.34 ^a	±0.33	162.37 ^a ±1.87
	750	162.44 ^a	162.47 ^a	162.37 ^a		162.42 ^a ±1.87
	Over all mean	162.44 ^a	162.39 ^a	162.35 ^a	±2.29	
K mmol/l	0	6.98 ^a	6.95 ^a	6.90 ^a	±0.19	6.94 ^b ±0.11
	750	6.99 ^a	6.95 ^a	6.97 ^a		6.97 ^a ±0.11
	Over all mean	6.99 ^a	6.95 ^a	6.93 ^a	±0.13	
Osmotic pressure/Kg H ₂ O	0	334.25 ^a	323.71 ^{ab}	310.72 ^{bc}	±4.99	322.89 ^a ±2.96
	750	312.17 ^{bc}	312.10 ^{bc}	307.51 ^c		310.59 ^b ±±2.96
	Over all mean	323.21 ^a	317.90 ^{ab}	309.12 ^b	±3.63	

^{a,b,c} Means with different superscripts in the same row within item differ significantly (P<0.05)

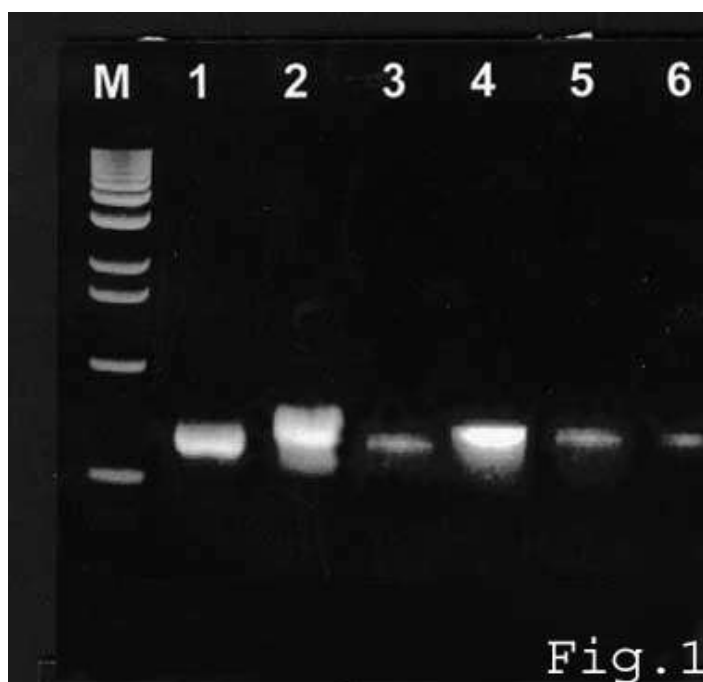
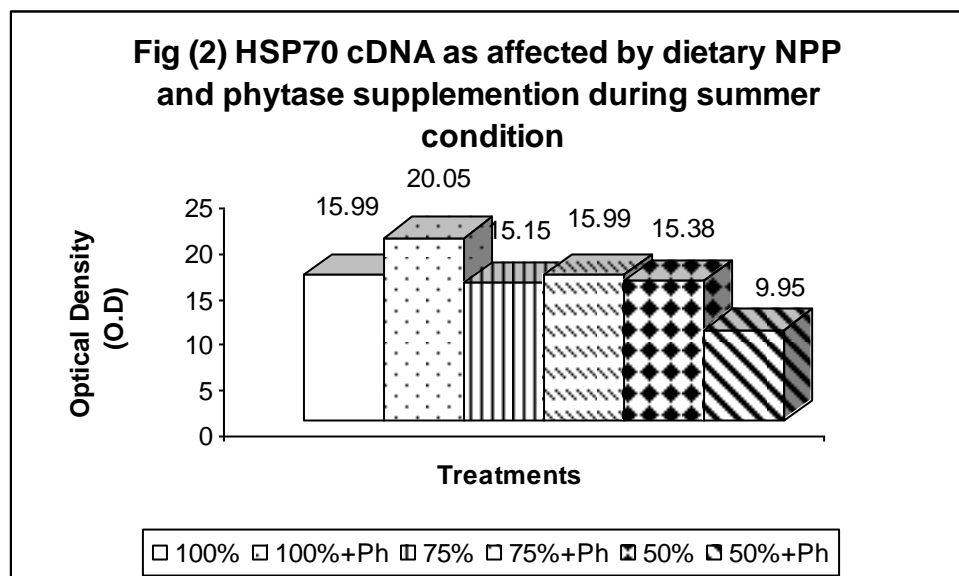


Fig (1) Gel electrophoresis of RT-PCR products of HSP70, Lane M represents DNA ladder, Lanes 1-6 represent PCR product single bands of an approximate size of 310 bp corresponding to HSP70 cDNA (1=100% NPP, 2=100% NPP+ phytase, 3=75% NPP, 4=75% NPP + phytase, 5=50% NPP, 6= 50% NPP + phytase)



REFERENCES

- Arad, Z.; and Skadhauge, E. 1984.** *Plasma hormones (Arginine Vasotocin, Prolactin, Aldosteron and Corticosteron) in relation to hydration state, NaCl intake and egg lying in fowls. J. Experiment. Zoo/.* 232: 707-714.
- Arad, Z.j Marder, J. and Eylath, U. 1983.** *Serum electrolyte and enzyme responses to heat stress and dehydration in the fowl (GALLUS DOMESTICUS). Comp.Biochem. Physiol* 74A(2): 449-451
- Aslam, S. M. 1995.** *Immune functions in broiler chicks as influenced by dietary phosphorus and vitamin D. Ph.D. dissertation. North Carolina State University, Raleigh*
- Attia, Y. A.; Qota, E. M. A.; Aggor, F. A. M. and Kies, A. K. 2003.** *Values of rice bran, its maximal utilization and upgrading by phytase and other enzymes and diet formulation based on available amino acid for broiler chicks. Archiv fur Gelfugelkunde,* 67: 157
- Balnave, D.; and Gorman, I. 1993.** *A role for sodium bicarbonate supplements for growing broilers at high temperatures. Worlds Poultry Sci. J.* 49:236-241.
- Bogin, E. and Keller, P. 1987.** *Application of clinical biochemistry to medically relevant animal models and standarization and quality control in animal biochemistry. J. Clin. Chem. Clin. Biochem.* 25: 873 - 878.

- Brees, K. W.; Raup, T. J.; Bottje, W. G.; and Odom, T. W. 1989.** *Physiological responses of heat-stress broilers fed nicarbazin. Poultry Sci.* 68:428-434.
- Craig, E. A. 1985.** *The heat shock response. CRC Crit. Rev. Biochem.* 18: 239-280.
- Dean, J. A. 1960.** *Flame Photometry. published by McGraw-Hill Book Company, pp.271-283.*
- Duncan, D. B. 1955.** *Multiple Range and multiple F test. Biometrics, 11: 1-42.*
- Edens, F. W.; Hill, C. H. and Wang, S. 1992.** *Heat shock proteins response in phosphorus-deficient heat-stressed broiler chickens. Comp. Biochem. Physiol. B* 103: 827-831.
- EL-Deeb, M.; and Abou Elmaged, A. 2001.** *Effect of cyclic heat stress on voluntary water consumption, efficiency of feed utilization and thyroid activity of broiler chicks. Egypt. Poult. Sci., (21) :811-831.*
- EL-Gendy, E.; and Washburn, K.W. 1995.** *Genetic variation in body temperature and its response to short-term acute heat stress in broiler. Poult. Sci., 74: 225-230.*
- Erdman, J. W., Jr. 1979.** *Oilseed phytates: nutritional implications. J. American Oil Chemists Society* 56: 736-741.
- Etches, R. J.; John, T. M. and Verrinder Gibbins, A. M. 1995.** *Behavioral, physiological, neuroendocrine and molecular responses to heat stress in: Poultry production in hot climates. edited by N. .T. Dagher. published by CAB International.*
- Geraert, P. A.; Padilha, J. C. F.; and Guillaumen, S. 1996.** *Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens. 2- Biological and endocrine parameters. Br.J. Nutr.* 75:205-216.
- Gomorri, G. 1942.** *Determination of inorganic phosphorus in plasma. J. Laboratory. Clinical. Medical, 27:955.*
- Guyton, A. C. 1981.** *Text Book 1- Medical Physiology W.B. Saunders Company (sixth edition), London.*
- Hawkey, C. M. and Dennett, T. B. 1989.** *A Color Atlas of Comparative Veterinary matology. Wolf Publishing Limited, London, England.*
- Hunsaker, W.G. 1969.** *Species and sex differences in the percentage of*

plasma trapped in packed cell volume determination on avian blood. Poult. Sci., 48: 907-909.

Husdan, H. 1968. *Determination of creatinine in serum. Chin. Chern. 14: 222 -232.*

Lima, F. R.; Mendonca, Jr. C. X.; Alvarez, J. c.; Garzillo, J. M. F.; Ghion, E. and Leal, P. M. 1997. *Biological evaluation of commercial dicalcium phosphates as sources of available phosphorus for broiler chicks. Poult. Sci. 76: 1707-1711*

Lindquist, S. 1986. *The heat shock response. Annu. Rev. Biochem. 55: 1151-1191.*

Lindquist, S. and Graig, E. A. 1988. *The heat shock proteins. Annu. Rev. Genet. 22: 631-677.*

Lou-Hong Zing; Wu Jian Liang; Xu Song; Lu YuLi; Xu YaoXing; Xu ShaoChun; Lou, H.X.; Wu, JL; Xu S; Lu YL; Xu, Y.X. and Xu, S. C. 1997. *The effect of supplemental phytase on growth performance and phosphorus utilization of broiler chicks. Acta Agriculturae Zhejiangensis, 9: 260-265.*

Mahmoud, K. Z.; Edens, F. W.; Eisen, E. J. and Havenstein, G. B. 2004. *The effect of dietary phosphorus on heat shock protein mRNAs during acute heat stress in male broiler chickens (Gallus gallus). Comp. Biochem. Physiol PartC 137: 11-18.*

Maloyan, A. Palmon, A. and Horwitz, M. 1999. *Heat acclimation increase the basal HSP72 level and alters its production dynamics during heat stress. Am. J. Physiol. 276: R1505-R1515.*

Mervat S. E. Yossef, M. A. Abaza, H. M. Yakout, A. A. Abdalla, and Azza ELSebai.2001, *Influence of microbial phytase supplementation on performance parameters and blood constituents of Gimmiizah hens fed different dietary phosphorus levels. Egypt Poult. Sci. Vol (21) (II): (997 _ 1020).*

Namra, M.M.M. 2006. *Influence of using baker's yeast and microbial phytase in Japanese quail diets on productive performance and some physiological parameters. Egypt Poult. Sci., 26 (11): (579 - 607)*

NRC 1994. *Nutrient Requirements of Poultry. 9th ed. National Academy of Science, Washington, DC. USA.*

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.

Pescatore, A. J.; Canter, A. H.; Jackson, K; Johnson, T. H.; and Pfoff, K. 1990. *Influence of barley- based diets on egg cholesterol content and production of two strains of laying hens. Poultr:' Sci . 69(1): 183- 190.*

Pilaski, J. 1972. *Vergleichende untersuchungen uberden hemoglobin halt des hunhner and geschlecht. Arch Gelfugelk, 36: 70-77*

QIAGEN, 1999. *QIAamp RNA Blood Mini Handbook. For total RNA isolation from whole blood. Pp: 4-39.*

Reddy, N. R; Sathe, S. K. and Salunkhe, D.K 1982. *Phytates in legumes and cereals. pp. 1-92 In: "Advances in Food Research." C. O. Chichester. E. M. Mark and G. F. Steward (eds), Academic Press, NY*

Richmond, W. 1973. *Colorimetric method for the determination of plasma cholesterol Clin. Chem. 19: 1350-1356.*

Saiki, P. K; Gelf, S. A.; Gelf, D. H.; Stoffel, S. J.; Higuchi, R; Horn, G. T.; Mullis, K. B. and Erlich, H. A., 1988. *Primer-directed enzymatic amplification of DNA with a thermo stable DNA polymerase. Science. 239: 487-490.*

Sambrook, J.; Fritsch, E. and Maniatis, T., 1989. *Molecular cloning a Laboratory Manual, Cold Spring Harbor Lab Press, New York USA. Tietz, N. W. (1982). Fundamental of Clinical Chemistry Edition by Norbert Sanrdrs Company, Philadelphia, USA.*

SAS 2001. *Statistical Analysis System, User's Guide Version 8.2. Cary NC. USA. Lindquist. S.: and Craig, E. A., 1988.*

Scheideler, S. E.; and Ferket, P. R. 2000. *Phytase in broiler rations: effects on carcass yields and incidence of tibial dyschondroplasia. J. Appl.Poult. Res., 9: 468-475.*

Schlesinger, M. J. 1986. *Heat-stress proteins: the search for function. J. Cell BioI. 103: 321-325.*

Sebastian, S.; Touchbum, S. P.; Chavez, E. R.; and Lague, P. C. 1996. *Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci., 75: 1516-1523.*

Sebastian, S.; Touchburn, S. P. and Chavez, E. R 1998. *Implications of phytic acid and supplemental microbial phytase in poultry nutrition:*

a review. World's Poult. Sci. J.. 54: 27-47.

Siegel, H. S.; Drury, L.; and Patterson, W. C. 1974. *Blood parameters of broilers grown in plastic coops and on litter at two temperatures. Poultry Sci., 53: 1016 - 1024.*

Smith, M. O.; and Teeter, R. G. 1993. *Carbon dioxide, ammonium chloride, potassium chloride and performance of heat distressed broilers. J. Appl Poultry Res. 1: 61 - 66.*

Stroev, E.A. 1989. *Biochemistry, First Ed. MIR Publishers, Moscow.*

Sturkie, P.d. 1986. *"Avian Physiology". 4th ed. Springer - Verlag New York, Inc. U.S.A.*

Teeter, R. G.; Smith, M. O.; Owens, F. N.; Arp, S. C.; Sangiah, S.; and Breazile, J. E. 1985. *Chronic heat stress and respiratory alkalosis: occurrence and treatment in broiler chicks. Poult. Sci. 64: 1060-1061.*

Underwood, E. J. and Suttle, N. F. 1999. *Mineral Nutrition of Livestock. CAB International Chapter 5: 105-148.*

Wahba, N. 1969. *Review or Biochemistry. First Ed. Vol. 1 . El-Naser Modern Bookshop. Cairo.*

Wang, S. 1992. *Steroid modulation of gene expression for heat shock proteins in domestic chickens. Ph. D. Dissertation. The Graduate School. North Carolina State University Raleigh North Carolina. Pp: 27695.*

White, B. A. 1970. *Determination of serum (SGOT) glutamic oxaloacetic transaminas and glutamic pyruvic transaminas (SGPT) activity. Chemistry for Medical Technologist, 3th Ed., 610-660.*

Wootton, I.D. 1982. *Microanalysis in Medical Biochemistry. 6th ed. P.58 Churchill LTD. London.*

Yahay, S.; Straschnow, A.; Plavnik, I. and Hurwitz, S. 1997. *Blood system response of chickens to changes in environmental temperature. Poultry Sci., 76:627-633.*

الملخص العربي

دور اضافة انزيم الفيتيز الى علائق البط المسكوفى فى التنظيم الحرارى والاسموزى اثناء فصل الصيف

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**قسم بحوث تغذية الدواجن - معهد بحوث الانتاج الحيوانى - مركز البحوث الزراعية - وزارة
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اجريت هذه الدراسة فى محطة البط بالسرو التابعة لمعهد بحوث الانتاج الحيوانى - مركز
البحوث الزراعية - وزارة الزراعة فى الفترة من شهر يولية الى سبتمبر لسنة 2005 وذلك لدراسة
دور اضافة انزيم الفيتيز مع مستويات مختلفة من الفوسفور المتاح الى علائق البط المسكوفى اثناء
فصل الصيف على التعبير الجينى لبروتينات الصدمة الحرارية (HSP70) ودرجة حرارة الجسم
ومعدل التنفس وعلى بعض القياسات الفسيولوجية والمناعية والضغط السموزى لبلازما الدم وايضا
على وظائف الكبد والكلية.

استخدم فى هذه الدراسة 126 ككتوت من البط المسكوفى عمر يوم وقسمت الى ستة مجاميع
متساوية وتم تكوين ثلاثة علائق احتوت على (0.14 - 0.25 - 0.45 %) و (0.13 - 0.21 -
0.41 %) فوسفور متاح خلال الفترة من عمر يوم وحتى ثلاثة اسابيع من العمر ومن ثلاثة حتى
الاسبوع الحادى عشر من العمر على التوالى.

تم اضافة انزيم الفيتيز الى كل العلائق بمعدل (صفر - 750 وحدة / كجم علف) وكانت النتائج
كالآتى.

معدل التنفس و التعبير الجينى لبروتينات الصدمة الحرارية (HSP70) ارتفع معنويا مع زيادة
مستوى الفوسفور او اضافة انزيم الفيتيز بينما لم تتأثر درجة حرارة الجسم بمستوى الفوسفور او
اضافة انزيم الفيتيز.

ادت زيادة مستوى الفوسفور او اضافة انزيم الفيتيز الى تحسن معنوى فى وظائف الكبد والكلية
و ايضا تحسن الضغط السموزى لبلازما الدم تحسنا معنويا مع زيادة مستوى الفوسفور او اضافة
انزيم الفيتيز.

ايضا اظهرت النتائج ان زيادة مستوى الفوسفور او اضافة انزيم الفيتيز ليس لهما تأثيرا معنويا
على عدد كرات الدم الحمراء و البيضاء وكذلك نسبة المكونات الخلوية بالدم بينما تحسنت نسبة
الهيموجلوبين معنويا مع زيادة مستوى الفوسفور او اضافة انزيم الفيتيز.

نستخلص من هذه الدراسة ان اضافة انزيم الفيتيز اثناء فصل الصيف له تأثيرات مفيدة على
المكونات الخلوية بالدم وعلى بعض القياسات الفسيولوجية و الضغط السموزى لبلازما الدم.