*Effect of Phytase on Semen Quality in Adult Male Turkey

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Abstract

The present study has been conducted to examine the effect of micobial phytase supplimentation with diets on male reproductive performance in adult turkey tom. Forty adult turkey males (aged: 36 weeks, weighted: 3.755 ± 0.165 kg) have been assigned into two experimental groups (20 males each). First group has been fed on standard provinder for eight weeks and served as control, second group has been fed on standard provender supplemented with phytase (1000 PTU) for eight weeks and served as treated group. Two weeks intervals, semen has been collected from the males and semen analysis was qualified. After 8 weeks, male turkeys have been anesthetized and testis were removed, and testis weight and size were measured. The results of phytase treated groups revealed significant increase of testis weight and size compared with control. Semen evaluation of treated group recorded significant increase of ejaculate volume, sperm concentration, ejaculate sperm number, individual and mass sperm motility, and percentage of live sperm, whereas percentage of dead sperm and abnormal sperm recorded significant decrease in comparison with control. It can be concluded that dietary supplementation of microbial phytase has potent improvement role in reproductive performance of adult turkey tom.

Zoology **Classification QL 700-739.8**

Key words: Semen, Phytase. Reproduction, Tom.

***The research cited from MSc thesis of the first researcher**

turkey and its use in artifitial insemination program implication, the present study has been designed to examine the effect of microbial phytase supplementation on reproductive perfomance in turkey males.

Materials and Methods

Phytase enzyme: Phytase enzyme product has been purchased from Bio-Feed-Novozyme, Denmark and supplimented with food in a dose 1000 IPU/kg of diet weight (10).

Experimental animals: The present study has been carried out at the college of Veterinary Medicine, Al-Qadisiya University, during the period extended from January, 2014 to August, 2014, on adult male turkeys obtained from local market around Al-Diwaneya city. The males were reared in a conditioned room (five males/ squared meter) in an ambient temperature around 23-25 ºC and 10/14 light to dark ratio and fed on standard provinder and alfalfa as well as drinking water *ad libitum*. The males were trained and conditioned for semen collection through abdominal massage.

Experimental design: After adaptation for two weeks, forty adult male Turkeys (aged: 36 weeks, weighted: 3.755 ± 0.165 kg) have been assigned into two experimental groups (20 males each): first group has been fed on standard provinder for eight weeks and served as control, and second group has been fed on standard provender supplemented with phytase (1000 PTU) for eight weeks, and served as treated group. Two weeks interval, semen has been collected from the males and semen analysis was qualified. After 8 weeks, male turkeys have been anesthetized by intraperitoneal injection of 0.3 ml of ketamin and 0.1 ml of xylazin /200 g bw, sacrificed, and testis were removed, weighted and size were measured.

Semen collection: Semen samples have been collected from Turkey males using the method of abdominal massage, described by Burrows and Quinn (11). The testes of Turkey males is located at the dorsum. After doing the abdominal massage and the back over the testes to stimulate the copulatory organ (the phallus), the tail has been pushed forward

Introduction

Phytic acid is a nutritional non-enzymatic antioxidant and is considered to have potential roles in reducing oxidative stress and as a preservative in foods. Phytic acid is considered to be the major storage compound for phosphorus of most seeds and cereal grains and contributes about 1 to 7% of their dry weight. Storage ability of phytic acid has been proved by chelating the multivalent metal ions, such as zinc, calcium, and iron. The binding capacity in phytic acid can result from the poor absorption of insoluble salts from the gastrointestinal tract, which may results in poor bioavailability of these minerals (1). Since chelation ability of phytic acid involves many divalent minerals that are essential for the intracellular and extracellular enzymes activities, a consequence of excessive chelation could lead to serious metabolic disorders (2).

Phytase (EC 3.1.3.8 or EC 3.1.3.26); *myo*-inositol hexa-phosphate 3- or 6-phosphohydrolases; is an enzyme know to be act in degrading phytic acid. So the enzymatic degradation of phytate can be facilitated by the supplimentation of different natural sources of phytase produced in various hosts, such as fungi and bacteria. In the abscence of sufficient level of phytase in the digestive tract, as in monogastric animals, undigested phytic acid could excreted to the outside of the body (1). Therefore, the application of phytase in animal feed could lowers phytic acid levels and could also reduces feeding costs, since it may reduces the requirement for inorganic phosphorus supplementation (3,4) and may reduce pollution of the environment caused by fecal excretion of phosphorus (5). Phytase has been used commercially to increase the availability of phosphorus broiler chicken (6,7) and to increase the reproductive efficiency in roosters (8). On the other hand, as it has been reported that commercial turkey breeders have poor frtility (9), so the managing commercial broiler breeders to maximize fertility becomes more challenging. To improve fertility by increasing sperm concentration and viability, which could improve the low fertility of commerrcial

been evaluated immediately after semen collection. A drop of semen was placed on a warmed glass slide and observed with a light microscope using low power (X10, X40) without cover slip. The mass activity was graded (0 to ++++) (Sastry, 2002), where (0) means no sperm motile, (+) means sperms have no progressive movement, (++) means sperms have slow progressive movement, (+++) means sperms have moderate progressive movement, and (++++)means sperms have strong progressive movement.

Sperm count (Concentration): Sperm count has been carried out with a Neubauer hemocytometer. The semen was diluted with normal saline at 1:200; the hemcytometer was then filled with the diluted semen through the capillary action of the red blood cell pipette. The hemacytometer was then mounted and the sperm cells counted (12).

Sperm number: Total sperm number in the ejaculate has been calculated by multiplying the sperm concentration (No. of sperms/ml) by the volume of ejaculate (ml) (12).

Live and dead ratio: Live- dead sperms ratio has been determined using Nigrosin-eosin stain (10% nigrosin and 5% eosin). A live spermatozoa were found to be clear, meaning they have not absorbed the stains, whereas dead spermatozoa were stained pink which means theyhave absorbed eosin stain component (12).

Statistical Analysis: Results were expressed as mean ± standard error. Comparisons were performed using one way analysis of variance (ANOVA1) and newman- keuls to test all groups unpaired values. Differences were considered to be significant at the level of $P<0.05$. All statistical analysis were carried out using the GraphPad Prism (SAS Institute, Inc., USA).

Results

Clinical observations: During the experimental period, extended for eight weeks, control turkey males showed normal activity and body features, whereas microbial phytase treated turkey males revealed higher activity and tend to be more aggressive than control males and usually tried to mate other males. Clinical findings also revealed high quickly with one hand and pressure was applied on the area using the forefinger and thumb of the same hand in order to milk the semen from it's ducts. Semen was then collected using a small rubber pipette and transferred into calibrated cup-like tubes. Semen was collected from toms pooled once each two weeks for 8 weeks. After pooling, the semen samples were diluted (1:200) before quantitative and morphological evaluation.

Testis weight: The left testis from each male has been weighted (g) by the use of sensitive balance, and the pecentage of testis weight then has been calculated for each 100 g of body weight as follow: {Testis weight(%) = testis weight (g) / body weight (g) x100%}

Testis volume: The left testis volume (ml) of each male has been measured by perfusion of the testis in the calibrated cylinder containing normale saline (0.89 %). The volume was calculated as equal to the displacement of the normale saline.

Semen analysis: According to Sastry (12), semen samples have been analyzed to determine volume of ejaculate, colour, sperm concentration, sperm number. Individual and mass motility, morphology and live-dead ratio.

Volume of ejaculate: The volume of ejaculate has been determined by aspiration of ejaculate by the use of calibrated pipette and the the volume read.

Colour of the semen: Semen colour has been checked by the use of nacked eye and consistently it was either milky, yellowish or greyish in colour.

Morphology: Sperm morphology was examined for both unstained sperms and those stained with Nigrosin and Eosin stain. A drop of unstained semen sample has been dropped on a glass slide to make a smear and viewed under light microscope (x40). For the staining semen sample, two drops of semen sample and one drop of 10% Nigrosin stain was added together with two drops of 5% eosin in a vial. After mixing thoroughly and gently, a smear has been made from the mixture and viewed under light microscope (x100).

Sperm viability (individual and mass motility): Individual and mass motility has

bw). Testis of phytase treated male turkey recorded siginificant ($p<0.05$) higher testis weight (0.0590 ± 0.0024) compared with that recorded by control male turkey (0.0475 ± 0.0017).

water consumption compared with control males.

Testis weight: Figure (1) illustrate the effect of microbial phytase supplementation on mature male turkey testis weight (g/100g,

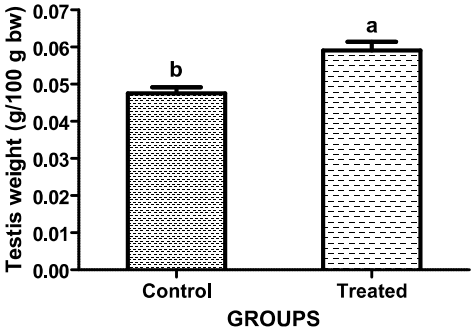

Figure (1): Effect of microbial phytase supplementation on testis weight (g/100g, bw) in mature male turkey. Values represent mean±standard error. Letter represent siginificant difference ($p<0.05$) between groups. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

Testis size: As illustrated in figure (2), mature male turkeys supplemented with microbial phytase recorded siginificant ($p<0.05$) increment of testis size (2.44 ± 0.123 ml) in comparison with that recorded by control mature male turkeys (1.850 ± 0.093 ml).

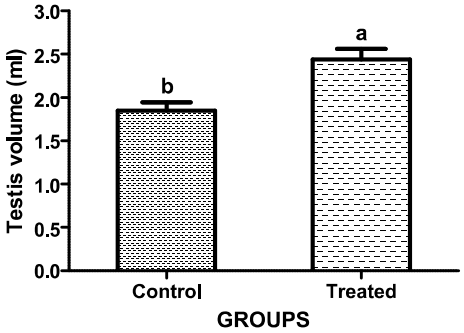

Figure (2): Effect of microbial phytase supplementation on testis size (ml) in mature male turkey. Values represent mean±standard error. Letter represent siginificant difference ($p<0.05$) between groups. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

control and treated groups, respectively), whereas the significant increment ($p<0.05$) of volume of ejaculate in microbial phytase treated group compared with control started on the 6th week of experiment (0.243 ± 0.0079 and 0.303 ± 0.0079 ml, for control and treated groups, respectively) and continued on the 8th week of experiment (0.251 ± 0.0088 and 0.313 ± 0.0082 ml, for control and treated groups, respectively).

Semen evaluation

1.Volume of ejaculate: Results clarified in figure (3), revealed no siginificant differences ($p>0.05$) in the volume of ejaculate (ml) in comparison between control and microbial phytase treated group on the 2nd week of the experiment (0.235 ± 0.0113 and 0.233 ± 0.0062 ml, for control and treated groups, respectively) and 4th week of the experiment (0.247 ± 0.0104 and 0.258 ± 0.0069 ml, for

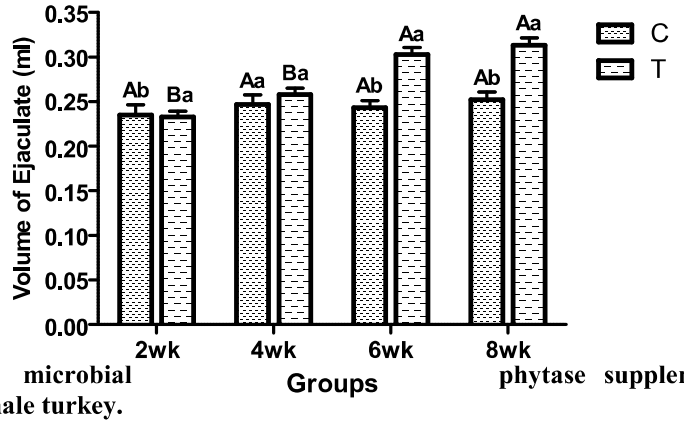

Figure (3): Effect of microbial phytase supplementation on volume of ejaculate (ml) in mature male turkey.
Values represent mean±standard error. Small letter represent siginificant difference (p<0.05) between groups. Capital letter represent siginificant difference (p<0.05) between periods. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

control groups on the 4th , 6th, and 8th weeks, respectively, whereas 2nd week recorded no significant difference between groups (3.327 ± 1.141 and 2.410 ± 0.803 for treated and control groups, respectively). In comparison between periods, the concentration in control group showed no difference (P>0.05) between each other, whereas treated group showed significant gradual increase as the period progressed.

2. Sperm concentration: Figure (4) demonstrates the result of sperm concentration ($x10^9$/ml) during the experimental periods. The significant (P<0.05) higher concentration has been recorded by microbial phytase group compared with control starting from 4th week of experiment and continued to the end of experiment (7.275 ± 1.282 and 3.510 ± 0.817, 8.700 ± 2.029 and 3.440 ± 0.850, and 3.560 ± 0.862, and 8.404 ± 1.891, for treated and

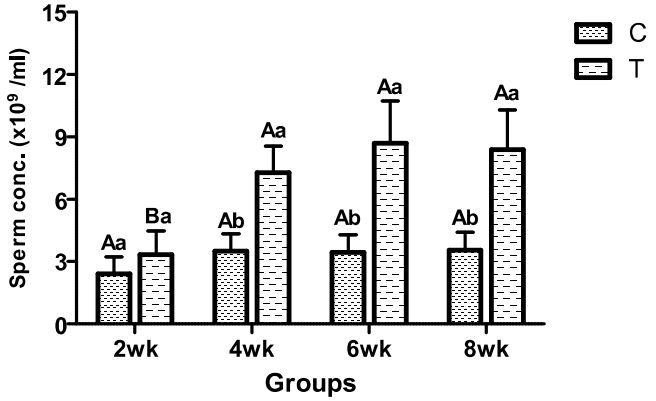

Figure (4): Effect of microbial phytase supplementation on sperm concentration ($x10^9$/ml) in mature male turkey.
Values represent mean±standard error. Small letter represent siginificant difference (p<0.05) between groups. Capital letter represent siginificant difference (p<0.05) between periods. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

that of microbial phytase treated males appeared milky and greyish in colour.

3. Semen colour: Semen of control turkey males tend to be yelowish in colour, whereas

control groups on the 4th , 6th, and 8th weeks, respectively, whereas 2nd week recorded no significant difference between groups (0.775 ± 0.374 and 0.566 ± 0.273 for treated and control groups, respectively). In comparison between periods, the concentration in control group showed no difference (P>0.05) between each other, whereas treated group showed significant gradual increase as the period progressed.

4. Sperm number: Figure (5) illustrates the result of sperm number (x10^9/ejaculate) during the experimental periods. The significant (P<0.05) higher concentration has been recorded by microbial phytase group compared with control starting from 4th week of experiment and continued to the end of experiment (1.877 ± 0.428 and 0.867 ± 0.297, 2.636 ± 0.672 and 0.836 ± 0.280, and 2.629 ± 0.618 and 0.897 ± 0.285, for treated and

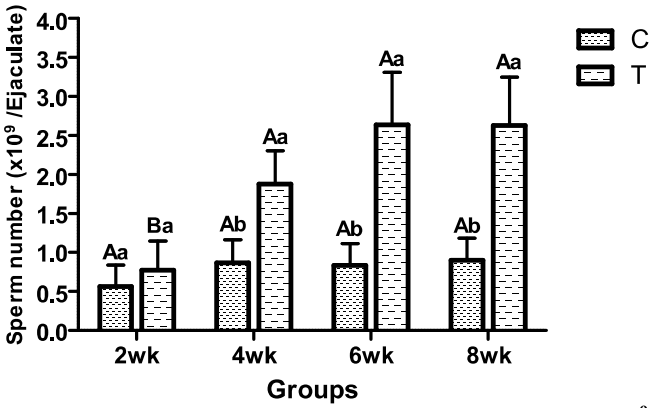

Figure (5): Effect of microbial phytase supplementation on sperm number (x10^9/ejaculate) in mature male turkey.

Values represent mean±standard error. Small letter represent siginificant difference (p<0.05) between groups. Capital letter represent siginificant difference (p<0.05) between periods. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

5. Individual sperm motility: The results illustrated in figure (6) showed the individual sperm motility (%) during the period of the experiment. In all experimental periods, the significant (P<0.05) higher concentration has been recorded by microbial phytase group compared with control (51.22 ± 3.485 and 27.11 ± 1.86, 63.83 ± 2.892 and 29.03 ± 2.733, 62.53 ± 3.573 and 25.18 ± 1.915, and 64.17 ± 2.987 and 26.22 ± 1.529), for treated and control groups on the 2nd , 4th , 6th, and 8th weeks, respectively. In comparison between periods, the concentration in control group showed no difference (P>0.05) between each other, whereas treated group showed significant increase on the 4th week of experiment and continued at the significant (P<0.05) higher level to the end of experiment, where the statistical analysis showed no significant differences (P>0.05) between the percentage of the 4th , 6th, and 8th weeks (table 1).

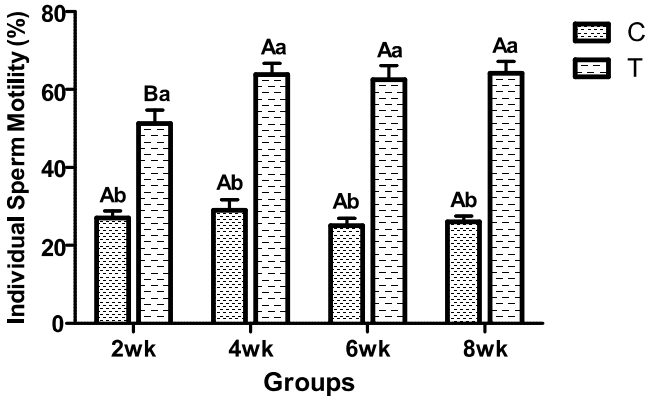

Figure (6): Effect of microbial phytase supplementation on individual sperm motility (%) in mature male turkey. Values represent mean±standard error. Small letter represent siginificant difference (p<0.05) between groups. Capital letter represent siginificant difference (p<0.05) between periods. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

Table (1): Effect of microbial phytase supplementation on individual sperm motility grades in male turkey.

No. of Animal	2nd week		4th week		6th week		8th week	
	%	Grade	%	Grade	%	Grade	%	Grade
T1	40	++	50	+++	50	+++	60	+++
T2	60	+++	70	++++	60	+++	70	++++
T3	50	+++	60	+++	60	+++	60	+++
T4	60	+++	60	+++	70	++++	60	+++
T5	60	+++	70	++++	60	+++	72	+++
T6	40	++	65	+++	75	++++	60	+++
T7	50	+++	60	+++	50	+++	60	+++
T8	40	++	70	++++	60	+++	60	+++
T9	57	+++	60	+++	70	++++	70	++++
T10	55	+++	70	++++	70	++++	70	++++
C1	20	+	20	+	20	+	20	+
C2	30	++	30	++	30	++	30	++
C3	30	++	30	++	30	++	20	+
C4	30	++	30	++	30	++	30	++
C5	20	+	30	++	20	+	20	+
C6	20	+	30	++	20	+	20	+
C7	30	++	30	++	30	+	30	++
C8	30	++	30	++	30	++	30	++
C9	20	+	30	++	20	+	20	+
C10	30	++	35	++	30	++	30	++

6. Mass sperm motility: The results illustrated in figure (7) showed the mass sperm motility (%) during the period of the experiment. The significant (P<0.05) higher concentration has been recorded by microbial phytase group compared with control starting from 4th week of experiment and continued to the end of experiment (52.50 ± 7.892 and 29.00 ± 3.733, 62.14 ± 8.573 and 31.23 ± 4.915, and 62.83 ± 7.987 and 30.70 ± 4.529, for treated and control groups on the 4th , 6th, and 8th weeks, respectively, whereas 2nd week recorded no significant difference between groups (35.83 ± 8.485 and 29.17 ± 2.860 for treated and control groups, respectively). In comparison between periods, the concentration in control showed no difference (P>0.05) between each other, whereas treated group showed significant gradual increase as the period progressed (table 2).

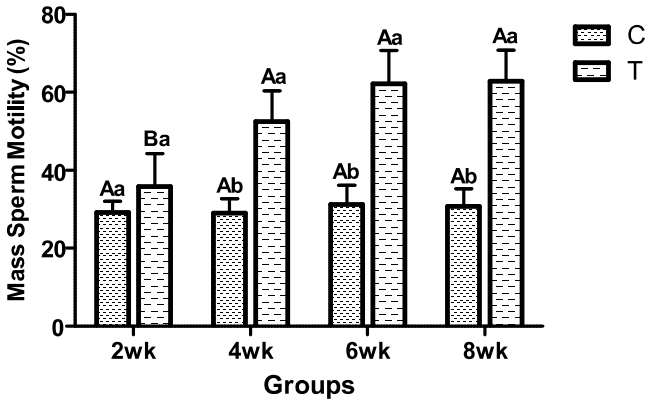

Figure (7): Effect of microbial phytase supplementation on mass sperm motility (%) in mature male turkey. Values represent mean±standard error. Small letter represent siginificant difference (p<0.05) between groups. Capital letter represent siginificant difference (p<0.05) between periods. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

Table (2): Effect of microbial phytase supplementation on mass sperm motility grades in mature male turkey.

No. of Animal	2nd week		4th week		6th week		8th week	
	%	Grade	%	Grade	%	Grade	%	Grade
T1	20	+	40	++	50	+++	50	+++
T2	40	++	60	+++	50	+++	60	+++
T3	40	++	50	+++	70	++++	70	++++
T4	40	++	70	++++	60	+++	60	+++
T5	40	++	40	++	50	+++	60	+++
T6	20	+	50	+++	70	++++	60	+++
T7	40	++	50	+++	70	++++	60	+++
T8	40	++	50	+++	60	+++	70	++++
T9	20	+	40	++	60	+++	70	++++
T10	50	+++	50	+++	75	++++	70	++++
C1	30	++	30	++	30	++	30	++
C2	30	++	30	++	30	++	30	++
C3	20	+	30	++	30	++	40	++
C4	30	++	20	+	20	+	30	++
C5	30	++	40	++	40	++	30	++
C6	30	++	20	+	27	+	20	+
C7	30	++	40	++	30	++	30	++
C8	36	++	30	++	30	++	30	++
C9	30	++	20	+	20	+	20	+
C10	30	++	30	++	55	+++	50	+++

7. Live sperm concentration

The results illustrated in figure (8) shows the percentage of live sperms (%) during the period of the experiment. The significant (P<0.05) higher percentage has been recorded by microbial phytase group compared with control starting from the 6th week of experiment and continued to the end of experiment (0.923 ± 0.0327 and 0.735 ± 0.0217, 0.917 ± 0.0321 and 0.749 ± 0.0216 for treated and control groups on the 6th, and 8th weeks, respectively), whereas 2nd and 4th week recorded no significant difference between groups (0.737 ± 0.0316 and 0.702 ± 0.0189, 0.769 ± 0.0319 and 0.722 ± 0.0221 for treated and control groups, respectively). In comparison between periods, the percentage in control group showed no difference (P>0.05) between each other,

whereas 2nd and 4th treated peroids showed no significant (P>0.05) differences between them, but 6th and 8th periods showed siginificant (P<0.05) increment compared with 2nd and 4th peroids.

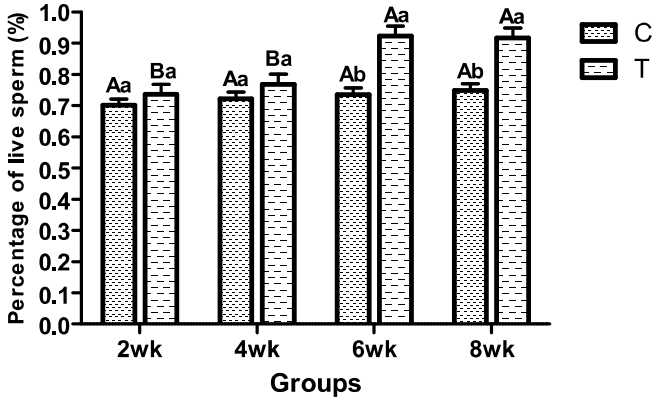

Figure (8): Effect of microbial phytase supplementation on live sperms (%) in mature male turkey.
Values represent mean±standard error. Small letter represent siginificant difference (p<0.05) between groups. Capital letter represent siginificant difference (p<0.05) between periods. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

6th, and 8th weeks, respectively), whereas 2nd and 4th week periods recorded no significant difference between groups (0.298 ± 0.0189 and 0.263 ± 0.0316, 0.278 ± 0.0221 and 0.231 ± 0.0319 for control and treated groups, respectively). In comparison between periods, the percentage of control group showed no difference (P>0.05) between each other, whereas 2nd and 4th treated peroids showed significant (P>0.05) higher percentage compared with treated 6th and 8th periods.

8. Dead sperm concentration

The results illustrated in figure (9) showed the percentage of dead sperms (%) during the period of the experiment. The significant (P<0.05) higher percentage has been recorded by control compared with microbial phytase treated group starting from 6th week of experiment and continued to the end of experiment (0.265 ± 0.0217 and 0.077 ± 0.0327, 0.251 ± 0.0216 and 0.088 ± 0.0331for control and treated groups on the

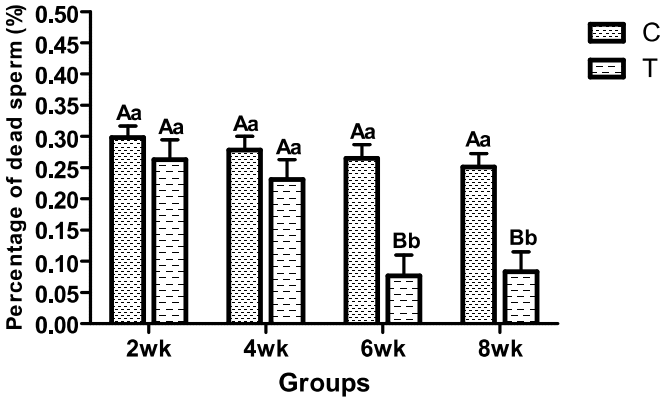

Figure (9): Effect of microbial phytase supplementation on dead sperms (%) in mature male turkey.
Values represent mean±standard error. Small letter represent siginificant difference (p<0.05) between groups. Capital letter represent siginificant difference (p<0.05) between periods. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

The results illustrated in figure (10) showed the percentage of abnormal sperms

9. Abnormal sperm concentration

control and treated groups (0.364 ± 0.0089 and 0.355 ± 0.016, 0.356 ± 0.022 and 0.371 ± 0.019 for control and treated groups, respectively). In comparison between periods, the percentage of abnormal sperms of control group showed no siginificant (P>0.05) differences between each other, whereas 2nd and 4th treated peroids showed significant (P<0.05) higher abnormal sperm percentage when compared with 6th and 8th treated periods.

(%) during the period of the experiment. The significant (P<0.05) higher abnormal sperms percentage recorded by controls group compared with microbial phytase treated group starting from 6th week of experiment and continued to the end of experiment (0.358 ± 0.017 and 0.302 ± 0.027, 0.344 ± 0.016 and 0.277 ± 0.021for control and treated groups on the 6th, and 8th weeks, respectively), whereas 2nd and 4th week recorded no significant (P>0.05) difference between

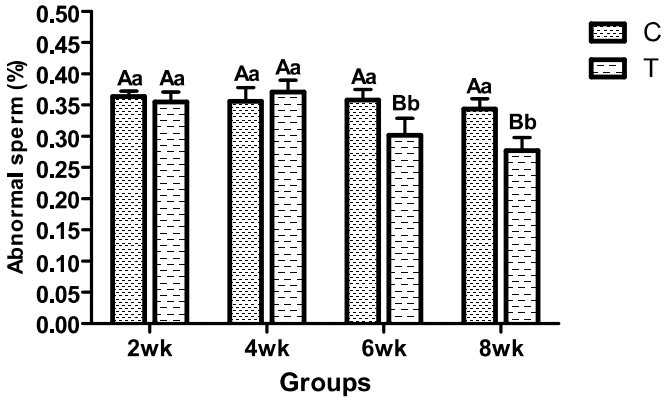

Figure (10): Effect of microbial phytase supplementation on abnormal sperms (%) in mature male turkey. Values represent mean±standard error. Small letter represent siginificant difference (p<0.05) between groups. Capital letter represent siginificant difference (p<0.05) between periods. Control group was drenched with empty capsule (vehicle) daily for 8 weeks. Treated group was fed on microbial phytase capsule (1000 PTU) daily for 8 weeks.

gradually increased as the treatment progressed. On the other hand, sperm abnormalities gradually decreased in microbial phytase treated turkey males compared with control males as the treatment progressed throughout the experimental period starting from 2nd week of the experiment (Figure 12).

10. Sprms density and abnormalities

Figure (11) clarify the effect of microbial phytase supplimentation on sperm density in the ejaculates at 2, 4, 6, and 8 weeks of the experimental period in mature turkey males. The result of treated males reveales higher density of sperm population compared control starting from 2nd week of treatment and

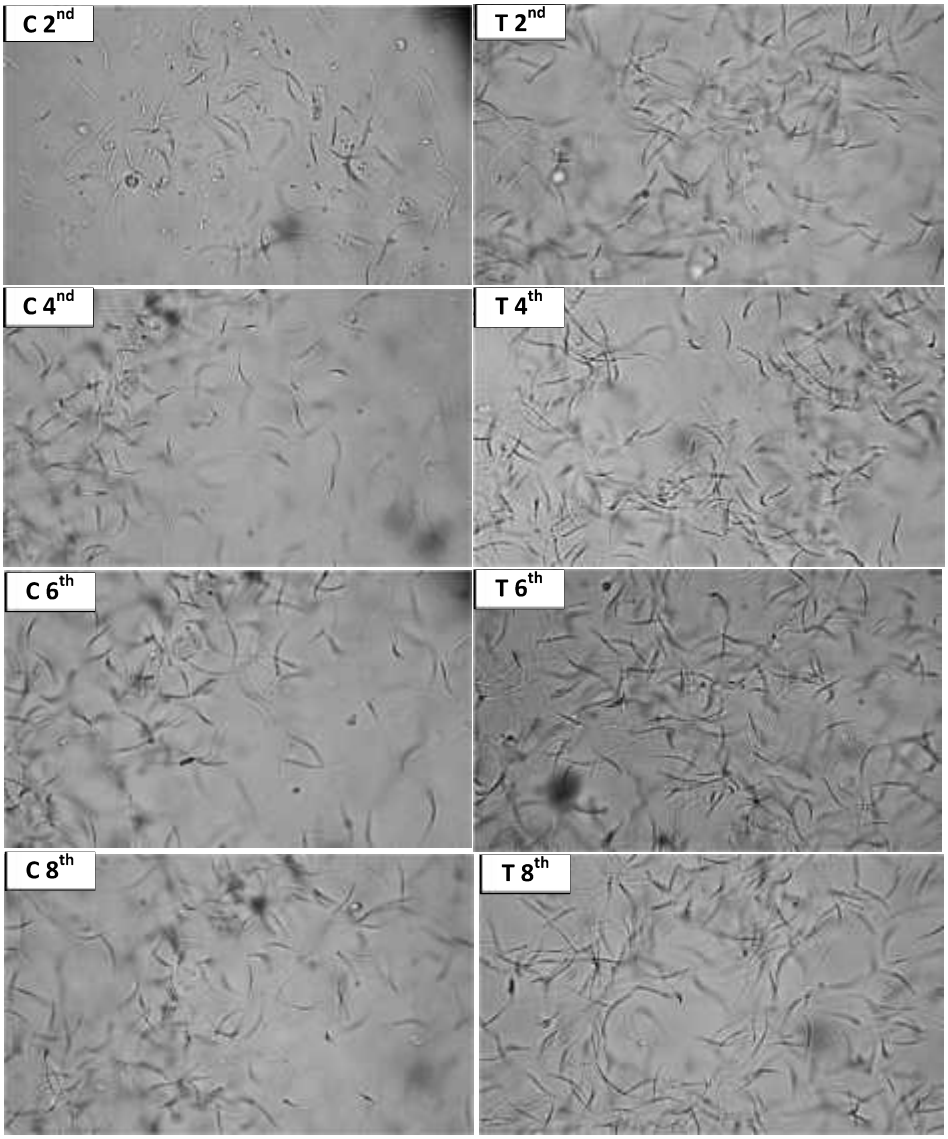

Figure (11): effect of microbial phytase supplimentation on sperm concentration in the diluted ejaculate (1:200) of adult male turkey after 2, 4, 6, and 8 weeks of treatment (x40). Where C represent intact control male, whereas T represent males supplimented with microbial phytase with diet (1000 FTU/kg diet).

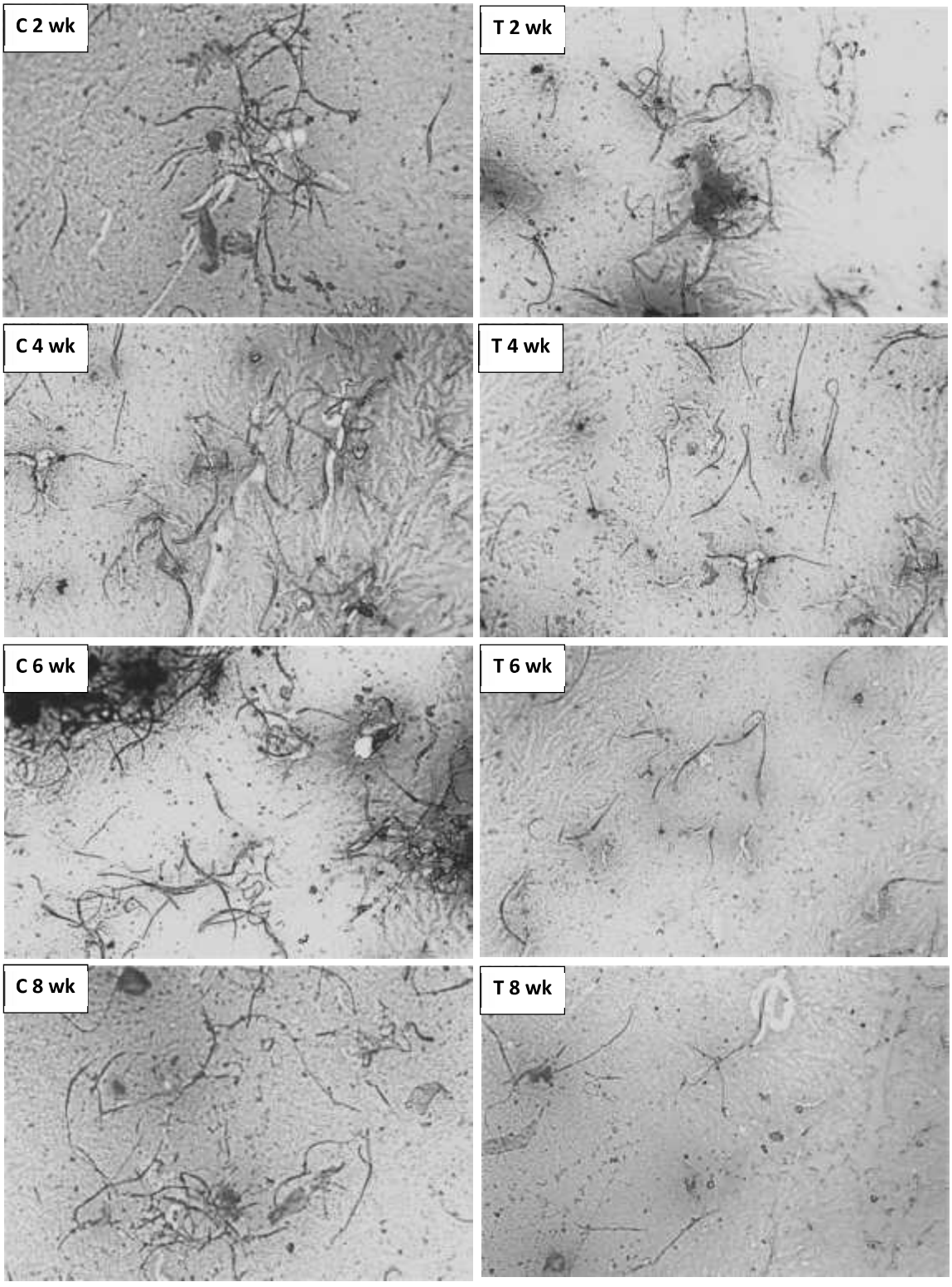

Figure (4-15): effect of microbial phytase supplimentation on dead sperm density in the diluted ejaculate (1:200) of adult male turkey after 2, 4, 6, and 8 weeks of treatment (x40). Where C represent intact control male, whereas T represent males supplimented with microbial phytase with diet (1000 FTU/kg diet) (E&N x500).

other hand, the use of dietary phytase could increase the metabolic status of spermatozoa which could provide a useful tool for increase of semen quality, because sufficient metabolism for energy production is one of the several attributes that a sperm must posses to fertilize an oocyte (20), and then to increase fertility aiming to predict male fertilizing ability, as spermatozoa should satisfy many requirements for successful fertilization (21), since bird spermatozoa are cells without intracytoplasmic energy reserves, and the turkey spermatozoa have low glycolytic activity (22), where could suggest the need for another metabolic pathway especially metabolism of fatty acids. In comparison with other minerals, phosphorus is the most important one to be considered in animal nutrition, where phosphorus has a key metabolic role and performe more physiological functions. These functions concern major metabolic processes and in utilization and transfer of energy as phosphorus plays an important part in energy regulation; in protein synthesis, transport of fatty acids and amino acid exchange, as phosphorus compounds are contributed directly or indirectly in all physiological functions such as intestinal absorption, carbohydrates oxidation, and contributes as a component of a large number of coenzymes; in growth and cell differentiation, where phosphorus is part of the nucleic acids structure, which are the site of genetic information and contributes in protein biosynthesis and immunity; in appetite control, efficiency of feed utilisation, and fertility (23). One of the important findings resulted in the present study was the improvement of semen quality in phytase treated turkey males in comparison with control. These positive alterations could be attributed to the role of microbial phytase in the release of different nutrients and amino acids and carbohydrates found in the dietary phytate (24,25,26) as well as the release of chelated minerals with phytate (27, 28). As it has been reported an increase of phytate digestibility after dietary phytase supplimentation (29,30,31). The presence of microbial phytase may act to decrease the

Discussion

The present results revealed improvement in the spermatogenic function of the testis due to microbial phtase treatmet as determined by increased testis volume, where Pierik *et al.* (13) reported that the spermatogenic function of the testis can be evaluated by determining testicular volume and total sperm count. The current testicular development can be attributed to the combined influence of hormones and proteins, including FSH, testosterone, inhibins, and activins, as they were detected in Sertoli, germ and Leydig cells throughout testis development (14). The present results showed gradual increase of testis volume as the treatment period progressed, thus additional suggestion can be kept in mind related to the increment of active mitotic division with spermatogonial maturation and accumulation of germ cells undergoing spermatogenesis leading to an increase in testicular size. This result was in agreement with Byrd *et al.*, (15) and Xia *et al*. (16) whom found increased testicular size due to the active mitotic division and accumulation of germ cells.

The current study recorded significant increase of sperm concentratin and total sperm number in the ejaculate in phytase treated turkey males in comparison with control. This increment could attributed to the increment of daily sperm production, which defined as the number of sperm produced per gram of testis per day (17).

The main purpose of the present study was to increase of male turkey fertility by improvement of motility, quantity and quality of sperm turkey by adding phytase to feed, where such application in poultry, phytic acid, that is abundant in all plant seeds, serve as the chief storage form of phosphorus. The ability of poultry to use phytate phosphorus is poor (18). Large portion of phosphorus in plant feed ingredients is present in the form of phytate, which is largely unavailable for nonruminant (19). In microbial phytase treated turkey males, it can be suggested that phytase role in providing the available phosphorus and increment of metabolism to produce the required energy, for both spermatogenesis and steroidogenesis. On the

essential to maintain the quality of the membrane that envelops the axial filament (41). These authors proposed that selenium played a direct role in spermatozoal structure, and its presence in the seminal plasma appeared to protect spermatozoal membranes from damage by metabolic free radicals, as it was found that the selenium content of testes and associated GSH-Px activity increased in the maturing rat (42).

It has been shown that microbial phytase treated turkey males revealed milky greyish to white in colour due, mainly, to the improvement of metabolism and intact epithelial cells within the efferent ducts. It has been recorded that the biochemical imbalances observed in semen from such roosters have been attributed to excurrent duct dysfunction (43).

According to Mann (44) considerable amount of ATP in spermatozoa formed during the later stages of spermatogenesis is required for good motility of spermatoza. Therefore, the present study could provide evidence that dietary microbial phytase supplimentation increased motility, quantity and quality of turkey males sperms. Supplimentation of phytase with diet, in the present study, provided an excellent nutritive and protective medium for spermatozoa on the basis that it provided higher concentrations of antioxidants. The observation that seminal plasma can protect spermatozoa against lipid peroxidation has been reported for mammalian semen (45). In avian species, there has also been evidence that seminal plasma can protect spermatozoa against peroxidation (46). The rate of oxidative metabolism in chicken spermatozoa was very similar to that in turkey spermatozoa although turkey spermatozoa were very dependent on oxidative metabolism to maintain optimal adenosine triphosphate (ATP) levels (47).

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effects of produced free radicals both during spermatogenesis and sperm transportation, since the different dietary phytase activities can increase the concentration of antioxidants in tje liver of broilers (32).

In the present study, it can be suggested that dietary phytase supplimentation could improve phytate phosphorus digestibility, which was in agreement with that reported by other researchers (6,7,33). Phosphorus is one of the important constituents that can be available after dietary phytase supplimentation, as phosphorus plays a very important biological role in livestock production and has more known functions than any other mineral nutrient (20, 34). In fact, phosphorus is involved in every cellular event in the body and in extracellular fluid while a vital role of phosphorus is as a structural element in the skeleton; phosphorus also plays important roles in many metabolic processes, lactogenesis and is important in the maintenance and growth (20, 35).

The amount of work invested in understanding the link between the tom and fertility is easily justified when evaluating the impact poor semen quality has on turkey production (36). The role of phytase in releasing of Zn from phytate is of important advantage, since Zn finger proteins continue to bind to DNA and regulate its transcription in all tissues. In addition Zn containing proteins are the receptors for steroid and thyroid hormones (37). On the other hand, Zn is a constituent or activator in hundreds of enzymes and is the most common mineral constituent in metalloenzymes (38). In addition, both Cu and Zn are required constituents for super oxide dismutase (SOD), as one of the important enzymatic antioxidant necessory for survive of spermatozoa (39). It has been found that dietary Zn supplimentation enhances spermatogenesis and improve semen quality (40).

Phytase, used in the present study as diet supplement, could release selenium chelated with the phytate. It has been found that selenium had aspecific role in spermatogenesis that could not be replaced by Vitamin E or any other antioxidant, as it has been hypothesized that selenium might be

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*تأثير الفايتيز في نوعية السائل المنوي لذكور التركي الناضجة

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الخلاصة

أجريت الدراسة الحالية بهدف اختبار تأثير الاضافة الغذائية للفايتيز المايكروبي على كفاءة ذكور دجاج الرومي التناسلية. تم توزيع 40 من ذكور الدجاج الرومي الناضجة (بعمر 36 اسبوعاً ووزن 3.755 ± 0.165 كغم) على مجموعتين متساويتين (20 ذكر لكل مجموعة). غذيت الأولى (السيطرة) على العليقة القياسية فقط لمدة 8 أسابيع وغذيت الثانية (المعاملة) على العليقة القياسية المزودة بأنزيم الفايتيز المايكروبي (1000 وحدة فايتيز دولية/ كغم من العليقة). تم جمع السائل المنوي كل اسبوعين لغرض التحليل المنوي. بعد مرور 8 أسابيع، تم تخدير ذكور الرومي وأزيلت منها الخصى وتم قياس أوزانها وأحجامها. بينت نتائج مجموعة المعاملة بالفايتيز زيادة معنوية في أحجام وأوزان الخصى بالمقارنة مع السيطرة. كما أظهر تحليل السائل المنوي لذكور المعاملة زيادة معنوية في كل من حجم القذفة وتركيز النطف وأعدادها في القذفة والحركتين الفردية والجماعية للنطف ونسبة النطف الحية في حين انخفضت معنوياً كل من نسبتي النطف الميتة والمشوهة بالمقارنة مع ذكور السيطرة. أظهرت ذكور مجموعة المعاملة كثافة أعلى في مجاميع النطف بالمقارنة مع السيطرة ابتداءً من الاسبوع الثاني من المعاملة واستمرت بالزيادة الى نهاية مدة التجربة. من جانب آخر، انخفضت أعداد النطف المشوهة في مجموعة المعاملة بالمقارنة مع السيطرة ابتداءً من الاسبوع الثاني ن مدة التجربة واستمر الانخفاض مع تقدم مدة الدراسة. يستنتج من الدراسة الحالية أن الاضافة الغذائية لانزيم الفايتيز المايكروبي لها دور فعال في تحسين كفاءة أداء الجهاز التناسلي لذكور الدجاج الرومي الناضجة.

الكلمات المفتاحية: السائل المنوي، الفايتيز، التكاثر، الدجاج التركي

*البحث مستل من رسالة ماجستير للباحث الأول