International Journal of Applied Poultry Research

ISSN: 2222-1263
www.ijscience.com

Zinc Oxide Supplementation Maintains Fertility of Feed-Restricted Turkey Toms

Faisal A Bughdadi

Department of Biology, University College, Umm Al-Qura University, Makkah, Saudi Arabia

ABSTRACT

In the present study, Zinc oxide supplementation in 15% restricted diet of turkey toms was used to monitor the effects on bird's fertility. Forty five turkey toms (16 weeks old) of an average weight 2.6 Kg were used and divided into three equal groups. Control group (C) ; birds fed on turkey breeder diet as 300 g/bird/day, Control restricted group (CR); birds fed on 15% restricted diet, 255g/bird/day and Zinc oxide supplemented group (ZnR); birds fed on restricted diet supplemented with 100 mg zinc oxide powder/Kg diet. Each group was offered the corresponding ration and water *adlibitum* for 3 months. At 4 weeks intervals, semen and testicular tissue samples were collected from 5 birds of each group (after slaughtering) to evaluate some testicular parameters including enzyme markers (acid phosphatase, alkaline phosphatase and lactic dehydrogenase), testicular total lipids, cholesterol, testosterone and estradiol 17β. Semen was first collected from control group 4 weeks prior to other groups (at 24 weeks of age), while it could be collected from other groups 4 weeks later. Semen evaluation showed a significant deterioration in sperm parameters (sperm count, viable sperm percentage, dead percentage and abnormal sperm percentage) of restricted birds. Activities of enzyme markers were significantly decreased at 24 and 28 weeks of age in birds fed on restricted diet. Meanwhile, no significant changes were recorded in control nor Zinc oxide supplemented birds. Total lipids and cholesterol were significantly increased in control restricted birds. Also, testosterone and estradiol 17β were significantly decreased in restricted birds. Supplementing Zinc oxide to restricted diet may be a saving practical tool for reducing cost of production without affecting fertility level (cost reduction=14.65%, putting into consideration that 1 kg diet costs 0.15 USD and 10 gm of Zinc oxide costs 0.05 USD).

Key words: Turkey toms; Fertility; Zinc oxide; Vitamin C; Feed restriction

Corresponding author: Faisal A. Bughdadi, Biology Department, University College, Umm Al-Qura University, PO Box 2064, Azizya, Makkah, Saudi Arabia.
Tel: + 966553515007; Fax: + 96625586531. E-mail : faisalbughdadi@hotmail.com

How to cite this article: Bughdadi FA, 2013. Zinc oxide supplementation maintains fertility of feed-restricted turkey toms. Int. J. Appl Poult. Res. 2(3): 33-37.

INTRODUCTION

Limiting the growth of male turkey breeders proportionally up to 0.35% during rearing, by feeding *ad libitum* a low protein diet, delays sexual maturity but has little effect on volume of semen, concentration of spermatozoa or final body weight (Hulet and Brody, 1986). However, if final body weight is limited to less than 0.6% of controls, semen volume and concentration of spermatozoa are decreased, and the proportion of males giving semen is reduced (Cecil, 1981).

Although there are a number of reports on the effects of feeding a low protein diet, there is little information on quantitatively restricting food intake during rearing of turkey males. In commercial broiler breeder flocks, male body weight during rearing is limited to about 0.5% of mature weight to optimize fertility. It is not easy to achieve such degree of body weight control satisfactorily by feeding low protein diets and therefore quantitative feed restriction become a normal commercial practice (Hocking *et al*., 1998).

For many years Zinc importance as an essential nutrient has been recognized. Recently, researchers have understood the full impact of this nutrient on animals and human health. They have identified over 200 Zinc-dependent enzymes in all the major biochemical pathways in the body (Case and Carlson, 2002). It is vital for the activity of a variety of hormones including glucagons, insulin, growth hormone and sex hormones. Key enzymes required for carbohydrate metabolism was reported to be lacking due to the reduced expression of zinc dependent messenger-RNA needed in the synthesis of such enzymes (O'Dell and Reeves, 1989). Meanwhile, in rams, spermatogenesis dropped to almost zero after 20 weeks on diet deficient in Zinc (White, 1993). The author reported that Zinc deficiency-induced anorexia reduced the secretion of gonadotropin-releasing hormone, a key regulator in ram.

The current study was designed to investigate the role of Zinc oxide as a feed supplement on some testicular fertility parameters in feed-restricted turkey toms.

MATERIALS AND METHODS

Experimental birds and management

The experiment was carried out partially at Physiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt. Measurements and data analysis were done at physiology Laboratory of Biology Department, University College, Umm-Al-qura University, Makkah, Saudia Arabia. Forty five growing male turkeys, with an average age 16 weeks old and weighing 2.5±0.25 kg were used in the experiment. Birds were divided into 3 equal groups (15 birds/group) as follows:

1. Group I (C)*:* served as control birds fed on a complete balanced commercial breeder turkey ration as 300 gm/bird/day (US Feed Grains Council, 1997).
2. Group II (CR)*:* fed on a complete balanced commercial breeder turkey ration with 15% restriction of daily requirement.
3. Group III (ZnR): fed on a complete balanced commercial breeder turkey ration with 15% restriction of daily requirement and supplemented with Zinc oxide powder at a dose equals to 100 mg/kg diet (Kim and Patterson, 2004). Each group of birds was fed on its corresponding ration for 12 weeks experimental period. Water and feed were offered *adlibitum* with a photoperiod 12 hours light and 12 hours dark.

Sampling and measurements

At 4 weeks intervals, semen samples and left testicular tissue samples were randomly collected from 5 birds per group after slaughtering and evisceration (Neuman *et al.,* 2002). Semen samples were quickly collected from the dilated terminal portion of ductus deferens (vas deferens) according to Saleh *et al*. (2004). The semen was diluted ten times with sterile warm isotonic sodium citrate solution (2.9%) to determine sperm cell count, percentage of viable, dead and abnormal sperms as preceded by Miller and Rass (1952).

Testicles were removed and washed with sterile ice cold isotonic saline (1.042%). Testes were kept at -20^{o}C for biochemical and enzymatic assays. Testicular homogenate was prepared according to the method adopted by El-Far (1996). Enzyme markers (acid phosphatase, alkaline phosphatase and lactate dehydrogenase) were determined in the prepared testicular homogenate according to Babson and Read (1959), Roy (1970) and Allain (1973) respectively. Testicular extracts were prepared according to Younis (1979) and used for determination of testicular total lipids and total cholesterol according to Schmitt (1964) and Flegg (1973) respectively. Testosterone content (Jaffe and Behrman, 1974) and estradiol 17β (Xing *et al*., 1983) were also measured.

Statistical analysis

Analysis of variance and Duncan's multiple range test for the obtained data were performed according to Snedecor and Cochrane, (1980).

RESULTS AND DISCUSSION

In the present study, the effects of excess Zinc supplementation in the ration of growing turkey toms subjected to 15% restricted diet on some fertility parameters were investigated. Table 1 shows that at 20th and 24th weeks of age, no significant differences were recorded in the activity of testicular acid phosphatase of all experimental groups when compared with the control. However, a significant decrease in its activity was observed at 28 weeks of age in CR group, while that fed on restricted diet supplemented with zinc (ZnR) revealed the same activity as control group.

Table 1: Effects of feed restriction with or without Zinc oxide supplementation on testicular acid phosphatase in turkey toms.

Age	Groups			Significance
(weeks)	C	CR	ZnR	P(F)
20	$0.51^{a} \pm 0.04$	$0.52^{a} \pm 0.02$	$0.49^{a} \pm 0.02$	0.813
24	$0.44^{ab} \pm 0.04$	$0.66^{a} \pm 0.20$	$0.50^{a} \pm 0.06$	0.023
28	$0.95^{a} \pm 0.05$	$0.63^{c} \pm 0.02$	$0.90^{a} \pm 0.04$	<0.0001

C: Control group; CR: Control restricted group; ZnR: restricted group with zinc supplementation; Values are means ± SE. $^{a-c}$Means within rows having different superscript letters are significantly different at (P<0.05) according to Duncan's multiple range test.

Testicular acid phosphatase, an enzyme of lysosomal origin detectable in all germinal cells and its specific activity increases with the development of spermatocytes (Males and Turkington, 1971). Samarth *et al.* (2001) attributed the elevation in testicular acid phosphatase activity to the increase in the synthesis of new lysosomes. In consistent, the decreased testicular acid phosphatase activity observed in control restricted group (CR) in the present work may be attributed to the reduction in the synthesis of new lysosomal testicular acid phosphatase which might be associated with testicular degeneration. Moreover, Saleh *et al.* (2004) reported that the activity of the tubular acid phosphatase showed the highest activity in stage corresponding to the final maturation process of the spermatid. And hence, the non significant effect observed in feed restricted birds supplemented with Zinc (ZnR) might be related to the synthesis of new lysosomes either in testicular spermatocytes or mature sperms which in turns counteract the deteriorated effect exerted by feed restriction upon acid phosphatase activity, maintaining such activity within normal levels.

Table 2 indicates no significant differences in testicular alkaline phosphatase activity among different experimental groups at 20 weeks of age. In addition, its activity was significantly decreased at 24 and 28 weeks of age in birds of CR groups. However, alkaline phosphatase activity in ZnR birds did not show any alterations, if compared with that of control unrestricted birds (P<0.01).

Kumar *et al.* (2003) correlated a decrease in alkaline phosphatase activity with the state of germ cell population and the loss of sperms from testes as the acrosome system of sperm head was found to contain alkaline phosphatase. Moreover, the activity of alkaline phosphatase was recorded to be low in men with spermatogenic arrest (Saleh *et al*., 2004). Consistently, supplementing Zinc oxide to restricted diet maintained fertility level nearly similar to that of the control unrestricted. This was in agreement with the finding of Batra *et al*. (2001) who observed a significant improvement in the testicular alkaline phosphatase and Na+-K+ ATPase in rats given Zinc.

Table 2: Effects of feed restriction with or without Zinc oxide supplementation on testicular alkaline phosphatase in turkey toms

Age	Groups			Significance
(weeks)	C	CR	ZnR	P (F)
20	$1.41^{a} \pm 0.14$	$1.52^{a} \pm 0.03$	$1.50^{a} \pm 0.04$	0.724
24	$0.95^{a} \pm 0.04$	$0.39^{b} \pm 0.07$	$0.89^{a} \pm 0.04$	<0.0001
28	$4.45^{a} \pm 0.32$	$3.37^{b} \pm 0.15$	$4.21^{a} \pm 0.33$	<0.0001

C: Control group; CR: Control restricted group; ZnR: restricted group with zinc supplementation; Values are means ± SE: [a-b]Means within rows having different superscript letters are significantly different at (P<0.05) according to Duncan's multiple range test.

Table 3: Effects of feed restriction with or without Zinc oxide supplementation on testicular lactate dehydrogenase (LDH) in turkey toms

Age	Groups			Significance
(weeks)	C	CR	ZnR	P (F)
20	$0.74^{a} \pm 0.08$	$0.80^{a} \pm 0.02$	$0.70^{a} \pm 0.05$	0.485
24	$0.74^{a} \pm 0.04$	$0.26^{b} \pm 0.07$	$0.70^{a} \pm 0.00$	<0.0001
28	$2.51^{a} \pm 0.30$	$0.96^{b} \pm 0.04$	$2.35^{a} \pm 0.18$	<0.0001

C: Control group; CR:Control restricted group; ZnR: restricted group with zinc supplementation; Values are means ± SE ,[a-b]Means within rows having different superscript letters are significantly different at (P<0.05) according to Duncan's multiple range test.

Data analysis tabulated in Table 3 shows no significant alterations in testicular LDH activity of all experimental groups at 20 weeks of age, although, at 24 and 28 weeks of age, its activity was recorded to be significantly lower in CR group compared to control ones. No significant differences were detected in LDH activity between control and ZnR birds all over the experimental times.

Testicular Lactate dehydrogenase (LDH) was found to be located primarily in the cytosol with small proportions being localized in mitochondria and cell surface of spermatozoa (Alvarez and Storey, 1984). It was reported that the expression of LDH activity in premeiotic, meiotic and postmeiotic cells including spermatids and spermatozoa, both in avian and mammalin species (Arias *et al*., 2000). In the present study, the significant decrease in LDH activity observed in CR group , based on the evidence stating that lactate dehydrogenase activites in spermatogenic cells have higher affinities for lactate than those of somatic cells and the fact that LDH maintains germ cells *in vivo* through favoring ATP production by advanced spermatogenic cells via the conversion of lactate produced by Sertoli cells to pyruvate and subsequent mitochondrial oxidation (Li *et al.,* 1989), may be related to the degenerative effects induced by such group on testicular cells and the subsequent reduction of glycolytic substrates (lactate) and energy production. And consequently the improvement observed in the activity of lactate dehdyrogenase in ZnR group provides further evidence that zinc oxide maintains fertility levels of restricted birds within the normal ranges.

Table 4 shows that no significant differences were recorded among all experimental groups, concerning testicular total lipids and cholesterol at 20 weeks of age, while a significant increase in their contents were detected in the testicles of CR birds at 24 and 28 weeks of age, when compared with control and ZnR groups.

Cerolini *et al.* (2003) reported that lipids are a basic component of semen. In particular, they contribute to the structure of spermatozoan membrane and are involved in vital aspects of sperm metabolism and function. Lipids were supposed to be an energy substrate for sperm metabolism. Recently, lipids are involved not only in sperm energy metabolism (Resseguie and Hughes, 1984) but also in all the main events that lead to fertilization. In mammalian species, cholesterol depletion through the plasma membrane with a consequent decrease of the cholesterol/phospholipids ratio is the suggested molecular mechanism responsible for the capacitation transformation (Tesarik and Flechon, 1986). The present results indicate higher levels of testicular total lipids and cholesterol of CR birds. Such increased content of lipids and cholesterol might reflect impaired lipid metabolism and utilization by testicular tissue and consequently a decreased release of arachidonic acid, the building unit of steroidal hormone synthesis, which was reported to be released with lysophospholipids during the breakdown of different phospholipids classes (Roldan and Murase, 1994). Consistently, Johnson (1970) associated the higher levels of fertility in rams with the lowered levels of lipids and cholesterol in the testis.

Table 5 showed that at 20 weeks of age, no significant alterations were observed in testicular estradiol 17β or testosterone concentration among all experimental groups when compared to control. Moreover, a significant decrease in their levels was recorded in CR group at 24 and 28 weeks of age if compared with unrestricted control (C) group. However, no significant differences were recorded between C and ZnR.

Although, testicular total lipids and cholesterol were significantly increased in CR birds, testicular contents of testosterone and estradiol 17β in such group were significantly decreased. These results might suggest impaired lipid metabolism and utilization by testicular tissue that could be lead to its accumulation in the testis. In consistent, Yoshioka *et al*. (2004) attributed the accumulation of Leydig cells containing numerous large lipid granules (involuted Leydig cells) in the interstitium of atrophied testes to the incapability of these cells to synthesize testosterone and the subsequent accumulation of testosterone precursors in the form of lipid droplets.

Table 6 shows a significant deterioration in the spermogram (low sperm count, low viable sperm percentage, and higher dead sperm % with higher abnormal sperm percentage) of CR birds if compared with that of ZnR birds.

Feed restriction has been used commercially to control the body weight of male-line turkeys primarily to improve semen production and to reduce feed consumption, the prevalence of musculoskeletal lesions and weight related disorders (Hocking *et al.,* 1998). The obtained results revealed a delayed semen production in birds subjected to feed restriction or restricted diet supplemented with Zinc oxide. Hulet and Brody (1986) reported that the mean semen volume of the control males was significantly greater than the mean volumes of feed restricted male turkeys. The delayed semen production observed in the present study due to feed restriction was in agreement with the findings of Menge and Frobish (1976) but contrast with the observations of Meyer *et al.*

Table 4: Effects of feed restriction with or without Zinc oxide supplementation on testicular total lipids and total cholesterol in turkey toms

Age (weeks)	Lipids	Groups			Significance
		C	CR	ZnR	P (F)
20	T.lipids	$11.88^{a} \pm 1.26$	$12.40^{a} \pm 0.21$	$12.80^{a} \pm 0.02$	0.303
	T.cholesterol	$3.80^{a} \pm 0.40$	$3.40^{a} \pm 0.08$	$3.70^{a} \pm 0.08$	<0.0001
24	T. lipids	$10.55^{b} \pm 0.55$	$28.58^{a} \pm 0.33$	$12.67^{b} \pm 0.27$	<0.0001
	T. cholesterol	$2.04^{b} \pm 0.04$	$9.29^{a} \pm 0.33$	$2.54^{b} \pm 0.17$	<0.0001
28	T.lipids	$37.74^{b} \pm 1.46$	$62.87^{a} \pm 3.54$	$42.74^{b} \pm 2.46$	<0.0001
	T. cholesterol	$9.70^{b} \pm 0.84$	$18.21^{a} \pm 0.48$	$12.00^{b} \pm 0.69$	<0.0001

C: Control group; CR: Control restricted group; ZnR: restricted group with zinc supplementation; Values are means ± SE. $^{a-b}$Means within rows having different superscript letters are significantly different at (P<0.05) according to Duncan's multiple range test.

Table 5: Effects of feed restriction with or without Zinc oxide supplementation on testicular estradiol 17β and testosterone concentrations in turkey toms

Age (weeks)	Hormone	Groups			Significance
		C	CR	ZnR	P (F)
	Estradiol 17 β	$2.28^{ab} \pm .22$	$2.17^{b} \pm 0.03$	$2.60^{a} \pm 0.04$	0.485
	Testosterone	$0.74^{a} \pm 0.07$	$0.69^{a} \pm 0.01$	$0.70^{a} \pm 0.01$	0.752
24	Estradiol 17 β	$20.77^{a} \pm 0.96$	$8.97^{c} \pm 0.15$	$18.90^{b} \pm 0.26$	<0.0001
	Testosterone	$0.69^{a} \pm 0.07$	$0.40^{b} \pm 0.03$	$0.67^{a} \pm 0.01$	<0.0001
28	Estradiol 17 β	$0.69^{a} \pm 0.07$	$0.40^{b} \pm 0.03$	$0.67^{a} \pm 0.01$	<0.0001
	Testosterone	$2.83^{a} \pm 0.11$	$1.22^{b} \pm 0.22$	$2.66^{a} \pm 0.15$	<0.0001

C: Control group; CR:Control restricted group; ZnR: restricted group with zinc supplementation; Values are means ± SE. $^{a-c}$Means within rows having different superscript letters are significantly different at (P<0.05) according to Duncan's multiple range test.

Table 6: Effects of feed restriction with or without Zinc oxide supplementation on testicular spermogram in 28 weeks old turkey toms

Age (weeks)	Groups			Significance
	C	CR	ZnR	P (F)
Sperm count (cell/µl)	$6.06^{a} \pm 0.09$	$2.75^{b} \pm 0.29$	$5.66^{a} \pm 0.15$	<0.0001
Viability %	$72.65^{a} \pm 2.14$	$65.87^{b} \pm 0.73$	$70.65^{a} \pm 2.14$	<0.0001
Dead sperm %	$26.30^{c} \pm 1.09$	$34.05^{b} \pm 0.60$	$28.53^{c} \pm 1.78$	<0.0001
	$27.76^{bc} \pm 2.68$	$31.08^{b} \pm 0.50$	$25.70^{c} \pm 1.06$	<0.0001

C: Control group; CR: Control restricted group; ZnR: restricted group with zinc supplementation; Values are means ± SE; $^{a-c}$Means within rows having different superscript letters are significantly different at (P<0.05) according to Duncan's multiple range test.

(1980) and Cecil (1981). However, in the present situation, the addition of Zinc oxide did not affect the onset of semen production, the observation that suggests the dependence of semen production on multifactorial events rather than Zinc oxide. Further results revealed that feed restriction adversely affected seminal parameters including sperm count, viability percentage, dead sperm percentage and abnormal sperm percentage, which may be attributed to the restriction in one or more dietary elements required for adequate semen characteristics.

Although zinc oxide was found to decrease free radicals production and lipid peroxidation during steroidogenic pathways in testicular tissues through acting as co-factors for Cu-Zn superoxide dismutase and peroxide-metabolizing enzymes; catalase, glutathione peroxidase (Ebesunun *et al*., 2004). In the present study, the improvement observed in semen parameters of bird fed on restricted feed due to zinc supplementation might be attributed to the antioxidant properties of zinc, a second mechanism of action for zinc through which it enhances fertility.

Conclusion

It is clear that zinc supplementation to feed restricted birds could maintain a fertility level nearly similar to that of the control unrestricted birds. So, the use of Zinc oxide supplementation economically could be considered as a beneficial tool for maintaining sound healthy fertility parameters with reduced production costs by 14.65%, putting into consideration that 1 kg diet costs 0.15 USD. and 10 gm of Zinc oxide costs 0.05 USD.

Acknowledgments

The author would like to acknowledge Dr. kamal A. Attia Professor of Animal Physiology, Faculty of Veterinary Medicine,Cairo University for offering management, kind help and advices during the practical work of the study.

REFERENCES

Allain CC, 1973. Quantitative determination of lactate dehydrogenase in serum. Clin Chem, 19: 223-228.

Alvarez J and BT Storey, 1984. Assessment of cell damage caused by spontaneous lipid peroxidation in rabbit spermatozoa. Biol Reprod, 30: 323-31.

Arias WM, C Mezquita and J Mezquita, 2000. Expression of lactate dehydrogenases A and B during chicken spermatogenesis: characterization of testis specific transcripts. J Cell Biochem , 79: 15-27.

Babson A and AP Read, 1959. Colorimetric determination of acid phosphatase. Am J Clin Path, 32: 89-96.

Batra N, B Nehru and MP Bansal, 2001. Influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels'. J Appl Toxicol, 21: 507-12.

Case CL and MS Carlson, 2002. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J Anim Sci, 80: 1917-1925.

Cecil HE, 1981. Effects of dietary protein on body weight and reproductive performance of male turkeys. Poult Sci, 60: 1049-1055.

Cerolini S, F Pizzi, T Gliozzi, A Maldijian, L Zaniboni, and L Parodi, 2003. Lipid manipulation of chicken semen by dietary means and its relation to fertility: a review. World's Poult Sci J, 59: 65-75.

Ebesunun MO, BA Solademi, OB Shittu, JI Anetor, JA Onuegbu, JM Olisekodiaka, EO Agbedana and AA Onyeaghala , 2004. Plasma and semen ascorbic levels in spermatogenesis. West Afr J Med, 23: 290-3.

EL-far FI, 1996. Effect of some anti-rheumatics on some hormones and testicular functions of male albino rats. MVSc, Thesis submitted to Faculty of Veterinary Medicine, Physiology Department, Cairo university.

Flegg HM, 1973. Quantitative enzymatic colorimetric determination of total and HDL cholesterol in plasma. Clin Biochem, 10: 79-86.

Hocking PM, R Bernard and TJ Wess, 1998. Comparative development of antitrochanteric disease in male and female turkeys of a traditional line and a contemporary sire-line fed adlibitum or with restricted quantities of food. Res Vet Sci, 65: 29-32.

Hulet RM and TB Brody, 1986. Semen quality and fat accumulation in prepuberal and postpuberal male turkeys as affected by restricted feeding. Poultry Science, 65: 1972-1976.

Jaffe BM and NR Behrman, 1974. Methods of hormone radioimmunoassay. Academic Press.

Johson AD, 1970. Testicular lipids, in: The testis (AD Johnson, WR Gomes and NL Vandemark eds), Vol. 2, Academic Press, New York, P: 193.

Kim WK and PH Patterson, 2004. Effects of dietary zinc supplementation on broiler performance and nitrogen loss from manure. Poult Sci, 83: 34-8.

Kumar M, MK Sharma, PS Saxena and A Kumar, 2003. Radioprotective effect of Panax ginseng on the phosphatases and lipid peroxidation level in testes of Swiss albino mice. Biol Pharm Bull, 26: 308-12.

Li SS, DA O'Brien, EW Hou, J Versola, DL Rockett and EM Eddy, 1989. Differential activity and synthesis of lactate dehydrogenase isozymes A (muscle), B (heart), and C (testis) in mouse spermatogenic cells. Biol Reprod, 40: 173-80.

Males JL and RW Turkington, 1971. Hormonal control of lysosomal enzymes during spermatogenesis in the rat. Endocrinol, 88: 579-89.

Menge H and LT Frobish, 1976. Dietary restrictions during adolescence and subsequent reproductive performance of turkey breeder males. Poult Sci, 55: 1724-31.

Meyer GB, CF Props, AT Leighton, HP Van Krey and LM Potter, 1980. Influence of dietary protein during the pre-breeder period on subsequent reproductive performance of large white turkeys. 3. The effects of semen volume and frequency of insemination on fertility and hatchability. Poult Sci, 59: 363-8.

Miller P and N Rass, 1952. Manual of infertility and artificial insemination in cattle. Balleière Tindall and Cox, London, UK, pp: 260-268.

Neuman SL, JI Orban, TL Lin, MA Latour and PY Hester, 2002. The effect of dietary ascorbic acid on semen traits and testis histology of male turkey breeders. Poult Sci, 81: 265-268.

O'Dell BL and PG Reeves, 1989. Zinc status and food intake, in: Zinc in Human Biology, ILSI Press, Washington, DC, P: 173.

Resseguie WD and BL Hughes, 1984. Phospholipid and cholesterol profiles from chicken seminal components during in vivo storage at 5□C. Poult Sci, 63: 1438-1443.

Roldan ER and T Murase, 1994 Polyphosphoinositide-derived diacylglycerol stimulates the hydrolysis of phosphatidylcholine by phospholipase C during exocytosis of the ram sperm acrosome. J Biol Chem, 269: 23583-23589.

Roy AV, 1970. Direct colorimetric determination of alkaline phosphatase. Clin Chem, 16: 431-439.

Saleh SY, KA Attia and FA Sawiress, 2004. Effects of melatonin supplement on some fertility parameters of turkey toms. 5th.International Symposium on Turkey Diseases, Berlin, 16th-19th June, Pp: 40-50.

Samarth RM, PK Goyal and A Kumar, 2001. Modulatory effect of Mentha piperita (Linn.) on serum phosphatases activity in Swiss albino mice against gamma irradiation. Indian J Exp Biol, 39: 479-82.

Schmitt JM, 1964. Colorimetric determination of total lipids with sulfophosphovanilic mixture. Thesis, Lyon.

Snedecor GW, WC Cochran, 1980. Statistical Method 7th ed Ames: Iowastate university press.

Tesarik J and JE Flechon, 1986. Distribution of sterols and anionic lipids in human sperm plasma membrane effects of in vitro capacitation. J Ultrastructure Molecular Structure Res, 97: 227-237.

US Feed Grains Council, 1997. Nutrition requirement of turkeys, in: Meat production from turkeys, ducks, geese and guinea fowls, P: 4.

White CL, 1993. The zinc requirements of grazing ruminants, in: Zinc in Soils and Plants: Developments in Plant and Soil Sciences (AD Robson ed), Vol 55, Kluwer Academic Publishers, London, P: 197.

Xing S, SZ Cekan and L Diczfalusy, 1983. Validation of radioimmunoassay for estradiol 17β by isotope dilution-mass spectrometry and by a test of radiochemical purity. Clin Chem Acta, 135: 189-196.

Yoshioka K, M Sasaki, S Imai, M Tsujio, K Taniguchi, and K Mutoh, 2004.Testicular atrophy after bile duct ligation in chickens. Vet Pathol, 41: 68-72.

Younis M, 1979. Effects of prolactin on synthesis, release and turnover of gonadotropins. PhD Vet Thesis, Cairo, University.