PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 10 (11): 1089-1093, 2011
ISSN 1680-5194

Influence of Dietary Supplementation with Zinc on Sex Hormones Concentrations of Broiler Breeder Chickens

Mahmood H.M. Amen[1] and Hazim J. Al-Daraji[2]
[1]Department of Animal Hygiene,
Kalar Technical Institute, Foundation of Technical Education-Sulaimaniya, Iraq
[2]Department of Animal Resources, College of Agriculture, University of Baghdad, Baghdad, Iraq

Abstract: This study was conducted to determine the effect of supplementing the diet with different levels of zinc (0, 50, 75, 100 mg/kg of diet) on sex hormones concentrations of broiler breeder chickens. A total of 132 (96 females and 36 males) of Cobb 500 broiler breeder chicken, 45 weeks old were used in this study. These birds were randomly distributed equally into four dietary treatments with three replicates each. Each treatment group constituted of 32 females and 12 males. Treatment groups were as following: T1: Birds fed the basal diet without any addition (0 Zn) (control), T2: Birds fed diet supplemented with 50 mg Zn (pure zinc)/kg of diet, T3: Birds fed diet supplemented with 75 mg Zn (pure zinc)/kg of diet and T4: Birds fed diet supplemented with 100 mg Zn (pure zinc)/kg of diet. Concentration of testosterone hormone in bird males was determined at 54, 58 and 66 weeks of age, whereas concentrations of estrogen and progesterone hormones in bird females were determined at 54 and 66 weeks of age. Results of this study indicated that adding zinc to diet (T2, T3 and T4) resulted in significant ($p<0.05$) increase respecting blood plasma concentrations of estrogen and progesterone hormones during 54 and 66 weeks of age as compared with control group (T1). However, dietary supplementation with zinc (T2, T3 and T4) caused significant ($p<0.05$) increase concerning blood plasma concentration of testosterone hormone at 54, 58 and 66 weeks of age and as regards the overall means of this hormone in comparison with control group. In conclusion feeding diet containing zinc resulted in significant improvement respecting sex hormones (testosterone, estrogen, progesterone). Therefore, adding ration with zinc can be used as a beneficial tool for improving reproductive performance of broiler breeder chicken.

Key words: Zinc, sex hormones, broiler breeder chicken

INTRODUCTION

In human, the zinc content of prostate gland, the seminal fluid and ejaculated sperm are very high and testicular zinc is essential for spermatogenesis (Vallee and Falchuk, 1993). The zinc content of sperm increases after exposure to seminal fluid, suggesting that sperms accumulate zinc as they traverse from the testicles to the urethra (Kvist and Bjorndahl, 1985). Zinc deficiency causes atrophy of the seminiferous tubules, failure of spermatogenesis,s and decreased testosterone secretion in the rat (Vallee and Falchuk, 1993). McClain *et al*. (1984) observed appropriate responses of pituitary Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) to Gonadotropin Releasing Hormone (GnRH) administration in zinc deficient rats. They concluded that the hypogonadism in zinc-deficient rats resulted mainly from leydig cell failure, but not from hypothalamo-pituitary dysfunction. Zinc deficiency impairs the responsiveness of leydig cell to gonadotropins and may cause primary hypogonadism in human as well as in experimental animals (Kaji, 2001). Hunt *et al*. (1992) also observed that there was decrease in serum testosterone concentration and seminal volume per ejaculate in healthy male volunteers fed zinc restricted diet for 35 days. Zinc is also very important for female reproductive function and necessary for normal ovulation and fertilization. Ronaghy and Halsted (1975) reported two female cases with short stature and hypogonadism, who were treated with oral zinc administration and attained catch-up growth and sexual development.

Zinc deficiency in pregnancy is associated with increased maternal morbidity, pregnancy related toxemia, spontaneous abortion, prolonged gestation or prematurity, in efficient labor, a tonic bleeding and increased risks to the fetus malformations and intrauterine growth retardation. These different effects of zinc can be explained by its multiple actions on the metabolism of sex steroids, together with prostoglandins (Favier, 1992). Zinc was shown to inhibit prolaction secretion from the anterior pituitary. Brandao-Neto *et al*. (1989) studied the response of plasma prolactin to oral zinc administration in healthy male and female adults and observed that prolactin concentration significantly decreased below basal levels in response to the increase in plasma zinc levels. Zinc is important

Corresponding Author: Hazim J. Al-Daraji, Department of Animal Resource, College of Agriculture, University of Baghdad, Iraq

for the cell division and the production of healthy sperm. It is the most critical trace mineral for male sexual function. It is needed for testosterone metabolism, testicle growth, sperm production, motility, count, reducing excess estrogen in male reproductive tissue. Zinc is needed for progesterone synthesis and a deficiency can produce excessive prolactin secretion (Brown and Pentland, 2007). A study has also shown that zinc helps in protecting the structure of the genetic material or the DNA chromatin in the sperm nucleus. This structure is important for successful fertilization (Balch, 2007). Zinc was shown to increase sex drive and male potency, reduce enlarged prostate, orally and typically relieve genital herpes (Natural Health Encyclopedia, 1998).

MATERIALS AND METHODS

This study was conducted to investigate the effect of dietary supplementation with different levels of zinc on concentrations of sex hormones in blood plasma of broiler breeder chicken. A total 132 (96 females and 36 males) of Cobb 500 broiler breeder chicken, 45 weeks old were used in this study. These birds were randomly distributed equally into four dietary treatments with three replicates each. Each treatment group constituted of 32 females and 12 males. Treatment groups were as following: T1: Birds fed the basal diet without any addition (0 Zn) (control), T2: Birds fed diet supplemented with 50 mg Zn (pure zinc)/kg of diet, T3: Birds fed diet supplemented with 75 mg Zn (pure zinc)/kg of diet and T4: Birds fed diet supplemented with 100 mg Zn (pure zinc)/kg of diet. Birds were raised on floors pens under similar environmental, managerial and veterinarian conditions. Birds were kept in a closed house; artificial lighting and drinking by nipples were provided through the experimental period (22 weeks). Commercial ration were provided for birds during experimental period. Birds were maintained under 16 hr light and 8 hr dark and mean temperature of 18-21°C during the whole period of study. For measuring concentration of testosterone hormone in blood plasma of males, three blood samples from each male were taken by using heart puncture procedure (Fig. 1) (Al-Daraji *et al*., 2008) each 30 minutes from six males that selected randomly from each males treatments (T1, T2, T3 and T4) and three blood samples from each female were also collected each 30 min from 6 females in each of females treatments. Blood plasma samples were frozen at -25°C till analysis.

Testosterone determination: The quantitative determination of total testosterone concentration in plasma was done by Micro plate Enzyme Immunoassay using ELISA device and commercial kits from (Monobin Inc., USA) according to procedure was followed by the supported company.

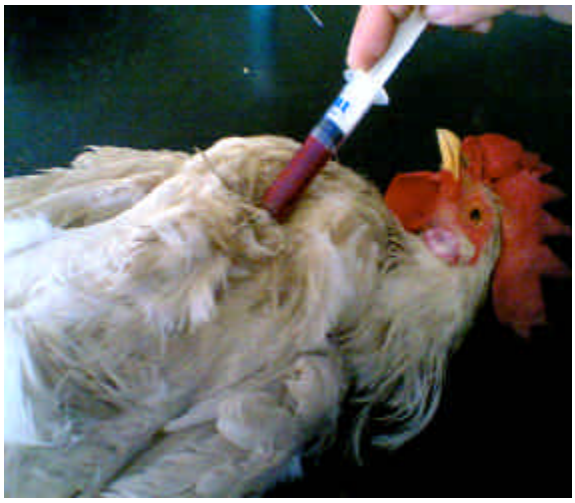

Fig. 1: Blood collection from birds by using heart puncture method

Estrogen determination: Enzyme Immunoassay for the quantitative determination of Estradiol (E2) concentration in blood plasma is a C18 steroid hormone with phenolic A ring. This steroid hormone has a molecular weight of 272.4. It is the most potent natural estrogen, produced mainly by the ovary and in smaller amounts by the adrenal cortex and the male testes (Tsang *et al*., 1980; Gore-Langton and Armstrong, 1988; Hall, 1988). Estradiol (E2) is secreted into the blood stream where 98% of it circulates bound to Sex Hormone Binding Globulin (SHBG). Esterogenic activity is affected via estradiol-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include the follicles, uterus, vagina, hypothalamus, pituitary and in lesser extent in the liver and skin. This test was conducted using (Biocheck. Inc) kit 323 Vintage Park Dr. Foster city, CA 94404, Cat. No. Bc-1111.

Progesterone determination: Progesterone hormone in blood plasma is measured by using kits supplied by (BioMerieux®Sa.Company) and VIDAS instrument. VIDAS progesterone is an automated test for use on the VIDAS instruments for the quantitative measurement of Progesterone (PRG), using the Enzyme Linked Fluorescent Assay (ELFA) technique. Progesterone is one of the main steroid hormones secreted by the ovaries (Franchimont *et al*., 1987). Its levels increases at the time of ovulation to reach its peak during the luteal phase (Hamers *et al*., 2001).

The data were analyzed statistically using the General Linear Models procedure of SAS (2000). Significant differences between treatment means are separated using the Duncan's multiple range test with 5% and 1% probability (Duncan, 1955).

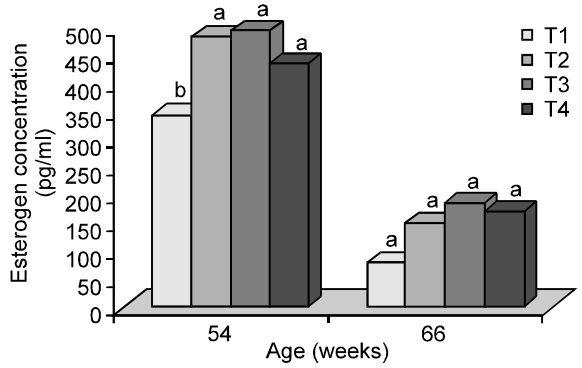

Fig. 2: Effect of dietary supplementation with zinc on estrogen concentration (pg/ml) in blood plasma of cobb500 broiler breeder females at 54 and 66 weeks of age. T1: Bird fed control diet (0 mg Zn/kg of diet) and T2, T3 and T4: represented birds fed diet supplemented with 50 mg Zn/kg of diet, 75 mg Zn/kg of diet and 100 mg Zn/kg of diet, respectively. [a,b]Bars within a week of age that lack common letters differ significantly ($p<0.05$)

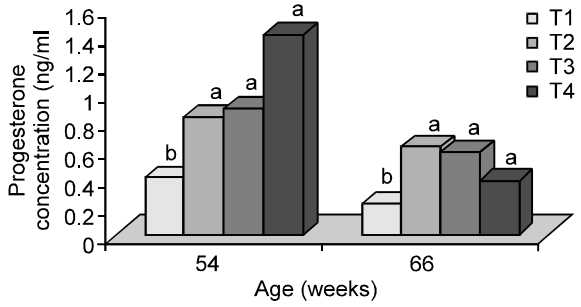

Fig. 3: Effect of dietary supplementation with zinc on progesterone concentration (ng/ml) in blood plasma of cobb 500 broiler breeder females at 54 and 66 week of age. T1: Bird fed control diet (0 mg Zn/kg of diet) and T2, T3 and T4: represented birds fed diet Supplemented with 50 mg Zn/kg of diet, 75 mg Zn/kg of diet and 100 mg Zn/kg of diet, respectively. [a,b]Bars within a week of age that lack common letters differ significantly ($p<0.05$)

RESULTS AND DISCUSSION

Female sex hormones: Results of this study revealed that the plasma esterogen concentration in birds of control group (T1) was significantly ($p<0.05$) lower than other experimental groups (T2, T3 and T4) at 54 and 66 wk of age (343.24 pg/ml) vs. (484.1, 495.37, 435.13 pg/ml, respectively) and (52.23 pg/ml) vs. (191.85, 224.98, 211.37 pg/ml, respectively). While, no significant differences in plasma estrogen concentration were observed among zinc treatments (T2, T3 and T4) throughout the period of this study (Fig. 2). Also, the plasma progesterone concentration in birds of control group (T1) was significantly ($p<0.05$) lower than other experimental groups (T2, T3 and T4) at 54 and 66 week of ages (0.406, 0.833, 0.896, 1.41 ng/ml, respectively) and (0.223, 0.623, 0.583, 0.383 ng/ml, respectively). However, no significant differences in plasma progesterone concentration were observed among zinc treatments (T2, T3 and T4) throughout the period of study. Moreover, T3 group (75 mg Zn/kg of diet) had the highest values as regards estrogen concentration (Fig. 3).

This significant decrease in estrogen and progesterone concentration in blood plasma of control group birds may be attributed to the decrease of LH and FSH release from the anterior pituitary gland and also may be due to progesterone acts at the level of the ovary and hypothalamus to stimulate the LH surge for ovulation. The progesterone is the major hormone secreted by granulose cells of large mature hierarchical follicles (F1) in birds and is produced by F1 follicle peaks 6 to 8 hr prior to ovulation (Johnson and Tienhoven, 1980) and triggers gonadotropin releasing hormone (GnRH) release by the hypothalamus. This in turn is followed by an increased in LH and FSH release from anterior pituitary.

The LH stimulates an even output of progesterone by the granulose cells of the F1 follicle (Etches, 1990, 2008), completing the positive feed back loop and producing the LH peak 4 to 6 h prior to ovulation (Johnson *et al*., 1985). It has been shown that chronic or acute injection of P4 may affect bird in a different manner (Zaghari *et al*., 2009). Administration of an acute dose of exogenous P4 has been shown to induce premature ovulation of a mature follicle at a specific time during ovulatory cycles in normal chicken hens (Nakada *et al*., 1994). Single injection of an acute dose of P4 in laying hens during preovulatory open-period has been shown to have positive effect on inducing preovulatory LH surge and ovulation (Wilson and Sharp, 1975, 1976; Johnson *et al*., 1985). However, chronic injection of P4 has been shown to increase baseline concentrations of P4 and result in arrested laying and disrupted distribution of hierarchical follicles in turkeys (Liu *et al*., 2001. Bacon and Liu, 2004). Progesterone acts on ovary and hypothalamus to stimulate the LH surge for ovulation. The rise progesterone concentration stimulates a rise in LH concentration, which in turn stimulates a further rise in progesterone. This positive feed back loop is what causes the progesterone and ultimately LH surge (Johnson *et al*., 1985). Zaghari *et al*. (2009) deduced that plasma estradiol (E2) concentrations were affected both by feeding pattern (*ad libitum* and restricted fed hens) and progesterone (P4) injection, also progesterone injection depressed ($p<0.001$) plasma E2 concentration in both feeding pattern. On the other hand, esterogen from the ovary stimulate the development of oviduct and

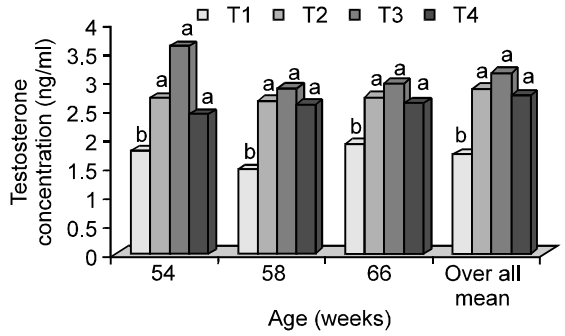

Fig. 4: Effect of dietary supplementation with zinc on testosterone concentration (ng/ml) in blood plasma of cobb500 broiler breeder males at 54, 58 and 66 weeks of age. T1: Bird fed control diet (0 mg Zn/kg of diet) and T2, T3 and T4: represented birds fed diet supplemented with 50 mg Zn/kg of diet, 75 mg Zn/kg of diet and 100 mg Zn/kg of diet, respectively. [a,b]Bars within a week of age that lack common letters differ significantly ($p<0.05$)

also caused an increase in blood calcium, proteins, fats, vitamins and other substances necessary for egg formulation. The public bones spread and the vent enlarges under the influence of estrogen (Card and Nesheim, 1973). Therefore, the significant amelioration of plasma estrogen concentration in birds fed diets supplemented with zinc may be attributed to the zinc role on the metabolism of proteins, amino acids, nucleonic acids, fat carbohydrates and vitamins as well as the metabolism of the other trace elements but also relates to the activity of prostaglandin, gonad stimulating hormone and it is necessary to the physiological function. Estrogen stimulates both vitellogenesis through its affect on liver and food intake (Al-Daraji, 2007b).

Male sex hormone: Results also revealed the superiority ($p<0.05$) of zinc treatment groups (T2, T3, T4) in comparison to control group (T1) after zinc was supplemented to broiler breeder males diets in plasma testosterone concentration on 54, 58 and 66 wks of age (Fig. 4). No significant differences were found among zinc treatment groups with respect to this parameter, while the differences between zinc treatment groups and control group were significant ($p<0.05$) on 54, 58 and 66 wks of age as well as regards the overall mean of this trait. From the results mentioned above, inclusion zinc to the diet of male broiler breeder significantly ameliorated testosterone concentration in blood plasma. Moreover, birds fed diets containing different levels of zing (T2, T3 and T4) recorded the higher overall mean testosterone concentration when compared with birds fed control diet (T1).

These significant increment ($p<0.05$) and amelioration in plasma testosterone concentration may be attributed to that the zinc is an inherent component of cortical substance of suprarenal gland. It is also participates in regulating the function of sexual gland system. The synthesis of testicosteroid depends on the existence of zinc. Zinc plays an important role on testosterone externalization through affecting the release of gonadotropic hormones (Fu-Yu *et al*., 2007). These results in the current study agree with the results of Fu-Yu *et al*. (2007) who found that testosterone in experimental groups was significantly higher ($p<0.05$) than that in control group. The improvement in plasma testosterone concentration may be also due to factors affecting the semen quality, such as the increase of relative weight and volume density of leydig cells (interstitial cells) which produce testosterone hormone. The interstitial cells produce several androgens, but the major hormone in blood is testosterone. The production of testosterone is stimulated by the rising blood concentrations of the gonadotroping, Luteinizing Hormone (LH) which stimulates the production of androgens from leydig cells of the testis. The blood concentration of LH is maintained by a negative feed back loop, in which elevated concentration of testosterone inhibited the secretion of Gonadotrophin Releasing Hormone (GnRH) which in turn inhibits the secretion of LH. As the secretion of LH declined, the concentration of androgens declines and hence the secretion of GnRH and LH is enhanced. In the absence of negative feed back in castrated males, the concentration of LH is 20-30 times greater than in intact males (Al-Daraji, 2007a; Etches, 2008). The testosterone concentration in serum and seminal plasma was positively correlated with the semen quality (Jianguo and Zhinian, 1990).

REFERENCES

Al-Daraji, H.J., 2007a. Artificial insemination in domestic birds. Ministry of Higher Education and Scientific Research, University of Baghdad, College of Agriculture.

Al-Daraji, H.J., 2007b. Avian Reproductive Physiology. Ministry of Higher Education and Scientific Research, University of Baghdad, College of Agriculture.

Al-Daraji, H.J., W.K. Al-Hayani and A.S. Al-Hassani, 2008. Avian Hematology. Ministry of Higher Education and Scientific Research, University of Baghdad, College of Agriculture.

Bacon, W.L. and H.K. Liu, 2004. Progesterone injection and egg production in turkey hens. Biol. Reprod., 71: 878-886.

Balch, J., 2007. Vitamins for a low sperm count. (www. Buzzle. Com.).

Brandao-Neto, J., B.B. de Mendonca and T. Shuhama, 1989. Zinc: An inhibitor of prolaction (PRL) secretion in humens. Horm. Metab. Res., 21: 203-206.

Brown, L. and S. Pentland, 2007. Health infertility organization: Male infertility-improving sperm quality. Acubalance wellness, Centre Ltd. Onwest 8th Ave. in Vancouver Bc. Canada.

Card, L.E. and M.C. Nesheim, 1973. Poultry production. 11th Edn., Lea and Febiger, Philadelphia, USA.

Duncan, D.B., 1955. Multible range and multiple F-test. Biometrics, 11: 1-42.

Etches, R.J., 1990. The ovulatory cycle of the hen, CRC Crit. Rev. Poult. Biol., pp: 293-318.

Etches, R.J., 2008. Reproduction in Poultry. Printed and bound in the UK.

Favier, A.E., 1992. The role of zinc in reproduction: Hormonal mechanisms. Biol. Trace. Elem. Res., 32: 363-382.

Franchimont, P., A. Demoulin and J.C. Valcke, 1987. Mechanismes endocriniens paracrines et autocrines du development folliculaire. Contraception-Fertilite-sexualite, 15: 479-492.

Fu-Yu, H. Ming-hai, L. Wen-Li, W. Ling-ling, S. Lie and Z. Ji-feng, 2007. Effect of different levels zinc on blood physiological and biochemical parameters in stud Holstein bulls. Chinese J. Anim. Nutr., 19: 5-12.

Gore-Langton, R.E. and D.T. Armstrong, 1988. Follicular steroidogenesis and its control. In: Knobil, E. and J. Neill, Ed. The Physiology of Reproduction. Raven Press, New York, pp: 331-385.

Hall, P.F., 1988. Testicular steroid synthesis: Organization and regulation. In: Knobil, E. and J. Neill, Ed. The Physiology of Reproduction, Raven Press, New York, pp: 975-998.

Hamers, N., J. Smitz and A. L'Hoir, 2001. Interet des controles externs de la qualite daus le suivi des performance des trouses illustre par un exemple du controle belge dansle domaine des immunoessais. Immunoanal. Biol. Spec., 16: 419-422.

Hunt, C.D., P.E. Johnson, J. Herbel and L.K. Mullen, 1992. Effects of dietary zinc depletion on seminal volume and zinc loss, serum testosterone concentrations and sperum morphology in young men. Am. J. Clin. Nute., 56: 148-157.

Jianguo, L. and I. Zhinian, 1990. Studies on the correlation between testosterone levels in serum and seminal plasma and semen quality. In 1 Stud bull. J. Agric. Univ. Hebei, 2: 14-21.

Johnson, A.L. and V.A. Tienhoven, 1980. Plasma concentrations of sex steroids and LH during the ovulatory cycle of the hen, *Gallus domesticus*. Biol. Reprod., 23: 386-393.

Johnson, P.A., Al-Johnson and V.T. Tienhoven, 1985. Evidence for a positive feed back interaction between progesterone and luteinizing hormone in the induction of ovulation in the hen, *Gallus domesticus*. Gen. Comp. Endocrinol., 58: 478-485.

Kaji, M., 2001. Zinc in Endocrinology. Int. Pediatrics, 16: 1-6, Shizuola Childrens Hospital, Japan.

Kvist, U. and L. Bjorndahl, 1985. Zinc presents an inherent capacity for human sperm chromatin decondensation. Acta Physiol. Scand., 124: 195-200.

Liu, H.K., D.W. Long and W.L. Bacon, 2001. Concentration change patterns of Luteinizing hormone and progesterone and distribution of hierarchical follicles in normal and arrested laying turkey hens. Poult. Sci., 80: 1509-1578.

McClain, C.J., J.S. Gavaler and D.H. Van-Thiel, 1984. Hypognadism in the zinc deficient rat: localization of the functional abnormalities. J. Lab. Clin. Med., 104: 1007-1015.

Nakada, T., Z. Koja and K. Tanaka, 1994. Effect of progesterone on ovulation in the hypo-physectomised hen. Br. Poult. Sci., 35: 153-156.

Natural Health Encyclopedia, 1998. www.Hotechno. Com.

Ronaghy, H.A. and J.A. Halsted, 1975. Zinc deficiency occurring in females. Report of two cases. Am. J. Clin. Nutr., 28: 831-836.

SAS, 2000. SAS/STAT User's Guide, Version 6.12. SAS. Inst. Inc., Tekirdag, Turkey, pp: 32-57.

Sauer, M.J., J.A. Foulkes, A. Worsfold and B.A. Morris, 1986. Use of progesterone. 11-glucuronide-alkaline phosphatase conjugate in a sensitive microtitre-plate enzyme immunoassay of progesterone in milk and its application to pregnancy testing in dairy cattle. J. Reprod. Fertil., 76: 375-391.

Tsang, B.K., D.T. Armstrong and J.F. Whitfield, 1980. Steroid biosynthesis by isolated human ovarion follicular cells *in vitro*. J. Clin. Endocrinol. Metab., 51: 1407-1411.

Vallee, B.L. and K.H. Falchuk, 1993. The biochemical basis of zinc physiology. Physiol. Rev., 73: 79-118.

Wilson, S.C. and P.J. Sharp, 1975. Changes in plasma concentration of luteinizing hormone after injection of progesterone at various times during ovulatory cycle of the domestic hen (*Gallus domesticus*). J. Endocrinol., 67: 59-70.

Wilson, S.C. and P.J. Sharp, 1976. The effect of progesterone on oviposition and ovulation in the domestic fowl (*Gallus domesticus*). Br. Poult. Sci., 17: 163-173.

Zaghari, M., R. Taherkani and S. Honarbakhsh, 2009. Effect of progesterone injection on performance, plasma hormones and ovarian morphology of *ad libitum* and restricted fed broiler breeder hens. Afr. J. Biotech., 8: 6481-6489.