EFFECTS OF RESTRAINT FEEDING AND HIGH DOSES OF ZINC ON OVARIAN STRUCTURE, HORMONAL PROFILE, INDUCTION OF MOLTING AND ZINC ACCUMULATION IN ORGAN TISSUES OF WHITE LEGHORN LAYER BIRDS

By

Latafat Amin Khan

FACULTY OF BIOLOGICAL SCIENCES
DEPARTMENT OF ANIMAL SCIENCES
QUAID-I-AZAM UNIVERSITY
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In the name of Allah

The most merciful and

The most Compassionate

'O' Allah open our eyes to see what is beautiful

Our minds to know what is true

Our hearts to love what is good

CERTIFICATE

This thesis, submitted by Latafat Amin Khan accepted in its present form the Department of Animal Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Doctor of Philosophy in Biology (Reproductive Physiology).

Supervisor: _____

Prof. Dr. Samina Jalali

External Examiner: _____

Dr.

External Examiner: _____

Dr.

Chairman: _____

Prof. Dr. Muhammad Shahab

Dated: _____

Dedicated to

MY PARENTS
My Mother

For her unconditional love encouragement, support

and her untiring concern for every aspect from beginning of my life

That I am capable of doing any thing I put in my mind

&

My Father

(May his soul rest in peace)

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LIST OF ABBRIVATIONS

Zn	Zinc
VFS	Visceral forebrain system
VIP	Vasoactive intestinal peptide
DOM	Day of molting
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
T_3	Ti-iodothyronine
T_4	Thyroxine
UEP	Universal Education Program
LH	Luteinizing hormone
FSH	Follicle stimulating hormone
TSH	Thyroid stimulating hormone
GnRH	Gonadotropins releasing hormone
ELISA	Enzyme linked immunosorbent assay
HCl	Hydrochloric acid
HNO_3	Nitric acid
ACTH	Adrenocorticotropic hormone
Cd	Cadmium
CAL	Calcium
BW	Body weight
GH	Growth hormone
CRF	Corticosteroids releasing factor
GRF	Growth hormone releasing factor
TRH	Thyrotrophic releasing factor
HDL	High-density lipoprotein
ZnO	Zinc oxide
ppm	Parts per million
Na	Sodium
KI	Potassium iodide
Al	Allmonium
HPA	Hypothalamic-pituitary-adrenal
DNA	Deoxyribo nucleic acid
CNS	Central nervous system
WHO	World Health Organization
HPG	Hypothalmus Pituitary gonadal axis
CS	Corticosterone
RNA	Ribonucleic acid

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LATAFAT AMIN KHAN
latafatk@yahoo.com

ABSTRACT

The present study was conducted to evaluate the effect of zinc oxides on reproductive structure and function in young laying birds (23 weeks of age) and old laying birds (65 weeks of age). Zinc is an important micronutrient in animal metabolism and in poultry. It is used as a dietary supplement to manipulate the reproductive system of the bird. For this purpose two experiments were conducted, Experiment-I and Experiment-II.

In Experiment-I, birds were divided in four groups. Treatment to these birds was given for 12 days. At 25 weeks of age five birds from each group were slaughtered. In the remaining five birds of each group treatment was withdrawn and they were kept on normal feed for a period of 15 days. The birds slaughtered after 12 days of treatment were designated as Batch-I and those slaughtered on 15^{th} day after the withdrawal of treatment were designated as Batch-II. In Batch-I before the start of treatment their initial body weight was recorded and after treatment body weight was recorded after every two days. Linear regression analysis of variance showed that zinc administration and off fed conditions caused significant decrease in body weight over the period of twelve days in all treatment groups. Mean comb length decreased significantly in Group-II (off fed), Group-III (25,000ppm Zn/Kg feed) and Group-IV (30,000ppm Zn/kg feed) compared to Group-I while comb width decreased only in Group-III. In Group-II (off fed) wattle length and width significantly reduced compared to that of Group-I. Mean ovarian weight and length reduced significantly in Group-II (off fed), Group-III (25,000ppm Zn/kg feed) and Group-IV (30,000ppm Zn/kg feed) compared to Group-I. Reduction in ovarian weight in Group-II was higher compared to zinc treated groups. Mean oviductal weight and length also decreased significantly in all treatment groups compared to Group-I. Oviductal length in Group-IV showed higher reduction compared to other two treatment groups (II & III). The liver weight of birds in Group-II (off fed) and Group-IV (30,000ppm Zn/kg feed) showed significant decrease, however, reduction in Group-II was greater compared to zinc treated groups. There was significantly higher zinc deposition in the ovary, liver and kidney of Group-III (25,000ppm zinc/Kg feed) and Group-IV (30,000ppm zinc/kg feed) but Group-II (off fed) showed no significant change compared to that of Group-I (control). The deposition of zinc in Group-IV was higher compared to Group-III. Significant reduction in mean plasma concentration of estradiol and progesterone in Group-II, Group-III and Group-IV was observed on 3^{rd}, 6^{th}, 9^{th} and 12^{th} day of experiment compared to the levels in Group-I (control). Linear regression analysis of variance showed highly significant decrease in plasma estradiol and progesterone concentration in Group-II (off fed), Zn treated Group-III (25,000ppm/Kg feed) and Group-IV (30,000ppm/Kg feed) over the period of 12 days. Mean plasma level of corticosterone started increasing significantly in Group-II, Group-III and Group-IV from 3^{rd} day of treatment and remained elevated on 6^{th}, 9^{th} and 12^{th} day of experiment compared to the level in Group-I (control). Linear regression analysis of variance showed highly significant increase in plasma corticosterone concentration in all treatment groups with the advance in the days of treatment.

In Batch-I mean number and diameter of yolky follicles reduced highly significantly in Group-II (off fed), Group-III (25,000ppm zinc/kg feed) and Group-IV (30,000ppm

zinc/kg feed) compared to the control. Due to the effect of these treatments large yolky follicles did not develop in treatment groups. Histomorphological measurements of ovarian follicular diameter showed highly significant reduction in all treatment groups, it was off fed group and high zinc treatment which had severe effect on reduction of follicular diameter in all categories above ≤200µm. Similarly, mean ovarian oocyte diameter significantly reduced due to feed withdrawal and two dosages of zinc. Compared to zinc treatments, feed withdrawal had more severe effect in reducing the ovarian oocyte diameter. Mean follicular wall thickness had also reduced significantly in all treatment groups but the effect was more severe in off fed and high zinc dosage group. Food withdrawal and high zinc dosage (30,000ppm zinc/kg feed) had highly significantly reduced the mean ovarian follicle number per section compared to Group-II (25,000ppm zinc/kg feed) in category ≤200µm. Group-II also showed significant decrease in mean ovarian follicle number in category 201-400µm compared to control group. Histological study showed less number of follicles which possessed unclear and shrunken nuclei, abnormal oocytes with disrupted ooplasm, disrupted zona pellucida layer, absence of demarcation between granulosa and basal lamina, reduction in thickness of follicular epithelium, loose thecal layer with less number of thecal gland and increased number of atretic follicles and stromal tissue arrangement with more interstitial spaces in all treatment groups compared to control group.

In Batch-II (Experiment-I) where birds were of 25 weeks of age and were slaughtered after 15 days of withdrawal of all treatments. Due to withdrawal of treatments, regression analysis of variance showed that mean body weight increased significantly in all the previously treated groups. In the case of secondary sexual characteristics comb and wattle length and width were comparable to the control, but in the low zinc treatments comb and wattle length and width increased significantly compared to control. Both in Batch-I and Batch-II zinc deposition in ovary was highly significant compared to the control. In contrast to Batch-I, in Batch-II there was no significant difference in zinc deposition in kidneys in treatment groups compared to the control. However, as with Batch-I, in Batch-II there was significant deposition of zinc in liver of treated groups compared to the control. Ovarian weight, length and width; oviduct weight and width and weight of liver responded differently after withdrawal of treatments. There was no statistical difference in ovarian weight in treatment groups compared to the controls. However, ovarian length in Group-II and Group-IV was significantly low compared to the control. Similarly, oviduct weight was significantly lower in Group-II and oviduct length was significantly low in Group-II and Group-IV compared to the control. Mean liver weight decreased significantly in Group-II and Group-IV.

In Batch-II (Experiment-I) withdrawal of treatments resulted in the increase of plasma estradiol and progesterone in all the treated groups, however, plasma progesterone levels increased to the level of control group. Linear regression analysis of variance showed significant increase in plasma progesterone and estradiol levels with the passage of time. In the case of corticosterone with advancing days of withdrawal of treatments linear regression analysis of variance showed highly significant decrease in concentration in all the three treatment groups. Mean ovarian yolky follicles number and size increased significantly in all the treated groups particularly small sized

ovarian yolky follicles and large sized yolky follicles also appeared, which were not present during treatment period. In the case of ovarian follicular diameter there was no significant difference in the treated groups and control birds. However, there was significant reduction in ovarian oocyte diameter category up to 801-1000µm and follicular wall thickness compared to the control. Perhaps, influence of the treatments persisted after withdrawal of the treatments. Re-feeding resulted in the increase in the mean number of ovarian follicles in all the treatment groups. Results of Batch-II showed that the stress of the treatments vanished due to withdrawal of the three treatments. Histological study of Batch-II showed increased number of follicles possessing very clear and spherical nuclei, normal oocytes with ooplasm, zona pellucida layer, very clear demarcation between granulosa and basal lamina, thickness of follicular epithelium improved, compact thecal layer with number of thecal gland and no atretic follicles. The stromal tissue arrangement also improved.

Experiment-II was started on the same pattern as that of Experiment-I with the difference that older birds of the age of 67 weeks were used. This experiment was also divided into Batch-I (in which 5 birds were slaughtered after 12th day of molting) and Batch-II (in which remaining 5 birds were slaughtered after 15 days of withdrawal of all the treatments).

In Batch-I (Experiment-II) there were four groups i.e. Control Group; Feed Withdrawal Group; Low zinc dosage (25,000ppmzinc/kg feed; High zinc dosage (30,000zinc/kg feed. During treatment period the same features, as with Experiment-I, were studied. Compared to the control, body weight was lost highly significantly in feed withdrawal group and both the groups treated with low and high zinc dosage.

Mean ovarian weight, length and width, oviduct weight and width as well as liver weight significantly reduced due to these treatments, significant deposition of zinc in ovary, liver and kidney was observed in zinc treatment groups. As in Experiment-I, plasma estradiol and progesterone levels highly significantly decreased. Linear regression analysis of variance showed highly significant decrease in plasma estradiol and progesterone concentration in all treatment groups over the period of 12 days. Corticosterone level increased significantly from 3rd day of treatment and remained elevated on 6th, 9th and 12th day of experiment compared to the level in Group-I (control). Linear regression analysis of variance showed highly significant increase in plasma corticosterone concentration in all treatment groups with the advance in the days of treatment. Mean ovarian yolky follicle number, ovarian yolky follicles diameter, ovarian follicular diameter, oocyte diameter and follicular wall thickness reduced significantly due to these three treatments. Histomorphological changes showed decrease in number of ovarian follicles, that were disintegrated and disrupted, loose thecal granulosa layer. Large number of atretic follicles, loose thecal layer, scanty thecal glands and disrupted tissue were observed. This was more pronounced in feed withdrawal group (off fed). Stromal tissue in treated groups was loosely arranged and lacunae were also observed.

When all the treatments were withdrawn (off fed, low and high zinc dosage), there was significant increase in body weight in all treated groups. Mean comb and wattle length and width increased in all groups during this period, however, in Group-II (off fed) it was still significantly less compared to control group. Ovarian and oviductal weight and length improved significantly. Ovarian length and width was significantly

low in Group-II and Group-IV. Similarly, oviductal weight and length also decreased significantly compared to Group-I. Linear regression analysis showed significant increase in estradiol and progesterone levels. There was highly significant decrease in plasma corticosterone concentration in Group-II, Group-III and Group-IV with the advance in the days of withdrawal of treatment. Ovarian yolky follicles number and diameter increased in all treatment groups. Ovarian follicular diameter, number, oocyte diameter and follicular wall thickness also increased significantly. There was significant improvement in histological changes were noted. Restoration of normal follicles, normal nuclei, zona pellucida attached to granulosa layer, and clear granulosa layer with basal lamina and thecal layer, normal thecal gland appeared as was seen in the control, stromal tissue was compact and no lacunae were seen. These results indicate that the treatment stress did not last long. This was neutralized as the treatments were withdrawn and normal feeding was restored. Molting lasted for 12 days. Egg production did not start but post-molting egg production was slow in the three treatment groups compared to the control. The lowest egg production in post-ovulation was seen in off fed group.

INTRODUCTION

IMPORTANCE OF ZINC

Zinc is an important micronutrient in animal metabolism. In poultry, zinc serves not only as a nutrient but can also be used as a dietary supplement to manipulate the reproductive system of the bird (Park et al., 2004d). The trace mineral Zinc is present in all living organisms and presents both structurally and catalytically in metalloenzymes. Zinc is required for the activity of over 300 enzymes which regulate many enzymatic and metabolic biochemical pathways in the body (Prasad, 1984; Prasad and Kucuk, 2002; Berger, 2003). In addition, zinc can influence gene expression by altering DNA and chromatin structure and is important for maintaining nucleic acid integrity (Castro and Sevall, 1993). These enzymes are vital to the activity of a variety of hormones including glucagon. Zinc enzymes assist in the synthesis of nucleic acids, mediate a number of physiological functions as well, including various immune responses and metabolism of hormones (Anonymous, 1988). It also plays a key role in the immune system, involved in bone formation, keratogenesis, reproduction, growth, wound healing, brain development and normal functioning of the central nervous system (Underwood, 1977; Vallee and Auld, 1980; Forbes, 1984; Spears, 1989; Wedekind et al., 1992; Hahn and Baker, 1993; Berger, 2003; Sahin et al., 2005). Zinc is found in the blood, kidney, pancreas, liver, prostrate, skin, bone, and eye. Among other functions Zinc affects bone development, feathering, enzyme structure and function, and appetite regulation in all avian species (Batal et al., 2001).

Currently, there are two inorganic feed-grade zinc added as a supplement to all formulated poultry diets commercially used by the poultry feed industry (Batal et al., 2001; Leeson and Summers, 1997; Wedekind and Baker, 1990) zinc oxide (ZnO 72% Zn) and zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$: 36% Zn). Supplemental zinc feed, 80–90% is ZnO, which is less bio-available for poultry than feed-grade Zn sulfate (Sandoval et al., 1997; Fosmire, 1990). The absorption, transport and utilization of vitamin A are also influenced by zinc status (Christian and West, 1998; McDowell, 1992). Zinc is absorbed in the small intestine and an intestinal pool of Zn formed by binding the metal to the intestinal metallothionein or Zn may be transported by

albumin in plasma to the liver thus normal concentration of vitamin A is maintained (Prasad, 1993; Berzin and Bauman, 1987). Burrell et al. (2004) reported improved performance when broilers consumed diets containing 110mg Zn per kg feed. Reduced chick mortality and improvement in broiler performance associated with organic Zn sources in broiler breeder (Hudson et al., 2005)

TOXICITY OF ZINC IN POULTRY

Although a bird requires a certain amount of zinc to remain healthy, high concentration of zinc is very toxic and can cause degeneration in the liver, kidney and pancreas and even death. There are two types of zinc poisoning: acute and chronic. (Highfill, 1998). Excessive dietary Zn intake disturbed blood lipid profiles, serum glucose and thyroid hormone (Chandra, 1984; Nishiyama et al., 1994). Dean et al (1991) observed decreasing levels of serum thyroxin (T4), triiodothyronine (T3), total cholesterol and high-density lipoprotein (HDL) cholesterol in broiler fed high doses of Zn whereas, plasma total cholesterol level remained unchanged (Samman and Roberts, 1988). However, the effects of excessive Zn on potential variation of endocrine and some blood metabolites in poultry are still to be investigated (Dean et al., 1991 and Kaya et al., 2001a).

High levels of zinc in the diet can result in reduced growth rates in chicks, cause ataxia, green diarrhea, erosion on gizzard and pancreas in laying hens (Dewar et al., 1983; Wight et al., 1986; Lu and Combs, Jr, 1988; Kazacos and Van Vleet, 1989; Anonymous, 1988)**,** high mortality in chicks (Blalock and Hill, 1988), reduced feed intake and egg production in laying hens and interfere with iron metabolism in chicks (Blalock and Hill, 1988; Hermayer et al., 1977; McCormick, 1984; Park et al., 2004c). Chronic Zinc toxicosis has also been associated with anemia in chickens, depression, intermittent lethargy, neurologic signs as seizures and increased thirst and urination (Lantzsch and Schenkel, 1978; Southern and Baker, 1983; Droual et al., 1991).

Zn toxicoses in the kidney include acute degeneration and necrosis of the proximal convoluted tubules and descending loops of Henle. (Breitschwerdt et al., 1986; Puschner et al., 1999; Annonymous, 1988).

ZINC DEFICIENCY

Zinc deficiency is characterized by reduced feed efficiency and utilization, thus leads to growth retardation, loss of appetite, hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, eye and skin lesions, and depresses immune function (Kidd et al., 1996; Shankar and Prasad, 1998; Prasad, 2004; Wang and Busbey, 2005; Maret and Sandstead, 2006). Even mild to moderate degrees of zinc deficiency impair macrophage and neutrophil functions, natural killer cell activity, and complement activity (Wintergerst et al., 2007; Anonymous, 2011). Zn deficiency also causes shortness and thickness in long bones of legs and wings, affects feather development, structure, scaling of the skin especially on the feet, and mortality in severe cases (Ensminger et al., 1990; Kidd et al., 1996; Hudson et al., 2005). Zinc deficiency retards the rate of healing of skin wounds, severe feet dermatitis and improves hoof health through keratin synthesis and epithelium maintenance in all species (Case and Carlson, 2002; Berger, 2003). Zinc deficiency instigates severe economic loss by reducing egg production, hatchability and embryos of Zn-deficient eggs have skeletal abnormalities (Selling et al., 1975; Case and Carlson, 2002; Berger, 2003). Sahin et al (2009a) reported improved feed conversion, egg production, egg shell weights and Haugh units when both Zn (30mg/kg) and pyridoxine (8mg/kg) were supplemented to laying hens. Zinc deficiency provokes oxidative damage through the effects of free radical action (Garfinkel, 1986; Powell et al., 1994; Salgueiro et al., 2000) and alters the status of antioxidant enzymes and substances (Prasad et al., 1993).

There are two common inorganic Zn supplements for poultry diets ZnO and ZnSO4 (Batal et al., 2001) but organic sources like Zn-methionine or Zn-propionate are more bioavailable to the chick than inorganic Zn sources (Spears, 1989; Wedekind et al., 1992; Hahn and Baker, 1993; Kidd et al., 1996; Sahin et al., 2009b). ZnO has an acute effect on the laying hen and this effect is reversible after removal. (Stevenson et al., 1987; Kaya et al., 2002). Deposition of Zn in chicks is linearly related to Zn levels in the diet (Sandoval et al., 1997; Kaya et al., 2001b; Bartlett and Smith, 2003).

USES OF ZINC SUPPLEMENTS

High concentration of Zn compounds in diet is considered as a method for the induction of a resting phase in laying birds (Creger and Scott, 1977 and Shippee et al., 1979). Hermayer et al. (1977) and Palafox and Ho-A (1980) documented that dietary Zn compounds, at levels up to 10 and 20g Zn/kg diet respectively caused a severe depression in food consumption, egg production and body-weight in adult female birds. Gentle et al. (1982) noticed in adult birds that ZnO supplementation at or above 6g Zn/kg diet produced a sharp decrease in the food intake. Jackson et al (1986) reported effects of high dietary levels of ZnO on the deposition of Zn in tissues, performance and tissue mineral accumulations in adult birds.

NATURAL MOLTING

Molting is a natural physiological process where the reproductive system of birds undergoes complete remodeling i.e. regression and rejuvenation and renew old feathers in preparation of organs for the next laying cycle (Berry 2003; McCowan, et.al., 2006; Anish, 2008 and Sundaresan et al., 2007). During molting hen puts the bulk of her energy into feather growth, leaving little for egg production. Natural molting is a seasonal process which occurs any time in birds when there are changes in day length or exposed to some stress such as lack of water or feed or lighting problems. When molting occurs in long-day periods it will be incomplete and hens may never be restored to full production. It is a good idea to allow hens to molt during winter by turning off the lights for complete molt (Hermes, 2003a).

In nature, all adult wild avian species undergo annual bird molting to renew their feathers as well as self-induced rest to rejuvenate body tissues and build up energy stores (Park et al., 2004d). The jungle fowl (*Gallus gallus*) is a wild ancestor of the laying hen, broodiness appears to be the primary initiating factor for domestic birds. In broodiness the jungle fowl hen experiences involution of the reproductive organs, stop of egg laying and eventually, the shedding and replacement of feathers (Sherry et al., 1980). Establishment of broodiness and incubation of her eggs is primary initiating factor for natural molting and continue for 21-28 days with little or no feed intake (Koelkebeck, 2001; Berry, 2003)

During this time, the hen experienced reduction of feed intake and body weight loses approximately 20-40% due to involution of the reproductive organs (Ankney and Mac Innes, 1978; Brake and Thaxton, 1979; Sherry et al**.,** 1980; Mrosovsky and Sherry, 1980; Etches, 1996). Etches (1996) reported that post-nuptial molt in non-domesticated birds occurs soon after the young are hatched and reared. These physiological changes cause the cessation of reproduction. Therefore, wild birds take a self-induced rest to rejuvenate body tissues to maintain flying capacity and build up energy stores to protect them from weather condition. (Park et al., 2004a; Faitarone et al., 2008a)

Birds in their natural habitat display molt twice a year, i.e., before and after reproduction (prenuptial or prealternate molt and a postnuptial or prebasic molt). However, domestic hens undergo molting at the end of their laying cycle (postnuptial), when the reproductive system also undergoes complete remodeling associated with feather replacement of tail, wing and body (Berry, 2003; Kuenzel, 2003). During this process, the reproductive tract regresses, rejuvenates, and prepares for a new cycle of egg production. (Sundaresan et al., 2008a)

A number of grouse, geese, ducks, quail, and pheasant species reduce food consumption accompanied by feather loss and regression of the reproductive system during incubation (Brake and Thaxton, 1979b; Mrosovsky and Sherry, 1980). The male emperor penguin (*Aptenodytes forsteri*) is responsible for incubating the sole egg laid by his mate during the Antarctic winter. During this time, spontaneous anorexia is observed in male because male did not move for feeding. The incubation period is about 120 days and during this period male loose 40% of their body weight (Le Maho, 1977; Mrosovsky and Sherry, 1980). From the above examples, it is evident that many bird species including the chicken survive with little or no food for relatively long periods of time because it is a normal feature of their physiology.

It is evident that a significant increase in metabolic rate, increases in whole body protein synthesis, osteoporosis, loss of body fat, and a suppression of the immune system occurs during this event of a bird's annual cycle. The VFS (visceral forebrain system) functions to regulate the balance of the autonomic nervous system. The VFS and VIP (vasoactive intestinal peptide) have been proposed to regulate the many behavioral and physiological events in the annual cycle of a bird, including

postnuptial molt. Therefore, the peptide may play a role in sensing and/or storing photoperiodic information. The peptide VIP is not only useful for identifying neural components of the avian VFS but also plays a functional role in mechanism involving long-day photostimulation and initiation of gonadal development. (Kuenzel, 2003). It is a normal feature of all birds, including chickens, to survive on little or no food for much longer durations and maintain themselves before returning from a non-productive to a productive cycle again (Berry, 2003). Migrating birds, especially shorebirds, fly up to 100h non-stop for thousands of kilometers without food or water and depend exclusively on body stores, despite their high metabolic rates (Butler and Woakes, 1990; Piersma and Baker, 2000; Battley et al., 2000).

Natural molt in most wild birds is associated with a decrease in plasma concentrations of estrogen and increase in plasma concentrations of tri-iodothyronine and thyroxin (Etches, 1996) In contrast to non-domesticated birds, most domesticated birds do not have the opportunity of molting because they are marketed after extended period of lay and never rear their young (Oguike et al., 2005a)

In commercial layer hens molting usually is incomplete because they continue to lay eggs at low rates for a prolonged period of time (Swanson and Bell, 1975ab; Berry, 2003 and Anonymous, 2010). An incomplete molt means a period of unprofitability due to a reduction of egg production and the end of the useful life of a flock (Berry, 2003; Dunkley et al., 2008ab).

In mammals, molting is combination of multiple factors i.e. a natural rhythm, hormonal levels and seasonal variation in light duration (Yousaf and Chaudhary, 2008). Snakes push back the skin around the lips by loosening and allowing it crawl out during molting. In Amphibians thyrotrophic hormones stimulate the thyroid gland to initiate the molting process. In arthropods, molting triggers the secretion of hormones by the epidermis, causing loosening and partial dissolving of the old cuticle, which splits, and allows the arthropod to emerge. Insects increase their linear dimensions at periodic intervals when the restricting exoskeleton is shed during molting. Mayflies spend up to three years as a ‘nymph’ stage, emerge from the water, usually at night, and molt (shed their skin) into a winged form and molt again into an adult mayfly. In crabs, the male and female molt at different times and the males mate with the females when the latter has shed their shells and are still soft. During molts

females become more attractive to the males. The growth of Lobsters results by periodic molting of their hard external skeleton about eight times in the first year, two to three times in the next two years and only once after 3rd year. Nematode worms undergo four molts between hatching and maturity (Burton and Burton, 1980).

RATIONALIZATION OF MOLTING

The practice of induced molting has been beneficial in extending the productive lives of birds which would otherwise be culled as soon as they reach a lower level of egg production. Molting involves reproductive quiescence (Burton and Burton, 1980; Venkata et al., 2008)

In commercial egg producer birds a period of unprofitably i.e. low egg production signifies the end of the useful life of a flock. At this most hens are sold and replaced just prior to the onset of natural molting. This generated interest in methods by which the natural molt could be avoided and flocks kept for more than one year. Forced molting means onset of artificial molting which occurs other than at the time of the natural molting. Egg production increased rapidly to a profitable rate following this artificial molt. Thus, the term "forced molting" was invented and came into common usage (North and Bell, 1999; Brake, 1993; Berry, 2003). In the commercial egg industry, different molt techniques are used before the end of the first laying to force hens before entering into a second egg laying cycle (North and Bell, 1999) for extending laying flock performance.

At the end of the laying cycle, egg production and quality decline significantly, leading some producers to induce a molt in the flocks in an attempt to improve performance. After the molting period, egg production and quality improve significantly compared to the premolt period. (Christmas et al., 1985; Zimmermann et al., 1987; Alodan and Mashaly 1999; Salem et al., 2005; Ebru Odabaşilar, 2006)

Induced molting is a process that simulates natural molting events. When birds return to full feed, a new plumage develops and the birds resume egg production at a higher rate with better egg quality. Induced molting extends the productive life of commercial chicken flocks and results in substantial reduction in the number of chickens needed to produce the nation's egg supply. Induced molting also has a positive impact on the environment through reduction of waste and natural resources

needed for growing more birds for egg production. However, molting induced by water deprivation or fasting causes discomfort and stress in hens (McCowan et al., 2006; Anonymous, 2010)

In addition to the economic benefits associated with additional egg laying cycles, molting provides the commercial egg producer litheness in the management of a flock to respond rapidly to shifts in the egg market as well as a change in feed and other input costs. Induced molting rejuvenates laying hens for a second or third cycle of production, resulting in higher egg production, heavier egg weight, and improvements in egg quality parameters, such as albumen height, shell thickness, and specific gravity in aged laying hens(Baker et al., 1981; Lee, 1982; Baker et al., 1983; Garlich et al., 1984, 1982; Berry and Brake, 1991; Al-Batshan et al., 1994; Heryanto et al., 1997a; Bell, 2003; Park et al., 2004b)

The technique of a stop or forced molt was recognized early in the century as a tool to improve performance and profitability of old laying hens. Egg production and quality decreases with advancement of age laying hens. Egg producers can impose an induced molt on older hens that results in increased egg productivity and decreased hen mortality compared with non-molted hens of the same age. (Golden et al., 2008)

However, despite the historical precedent for successful utilization of molting, controversy has come to the forefront in attempts to halt induced molts as they are currently practiced. (Park et al., 2004c)

BIOLOGICAL MECHANISMS OF INDUCED MOLTING

The ovulatory cycle and molting are physiological and endocrinological mechanisms involving a relationship of light stimulus, hypothalamus, pituitary, gonads, thyroid and adrenal glands but the relationships to the physiological mechanisms that induce postnuptial molting in non-domesticated birds are unknown (Etches, 1996). During ovulation, the LH surge triggers the release of a mature follicle. With advanced age of a laying hen, egg production decreases coinciding with decrease in ovarian steroids and gonadotropins. Molting occurs when estrogen, progesterone and luteinizing hormone are low while thyroid hormones and corticosterone are high. The physiological processes during induced molting are common to those found in natural molting (Oguike et al., 2005b).

The changes in reproductive functions during induced-molting were associated with reduced levels of LH and sex steroids (Decuypere and Verheyen, 1986; Jacquet et al., 1993). Molt induced by reduction in day length and starvation significantly decreases body weight and causes rapid rise in plasma levels of corticosterone as an evidence of physiological characteristic of stress (Wolford, 1984) as well as associated decline in sex steroids and gonadotropins (Oguike et al., 2005b).

During molting, plasma levels of LH, progesterone and estradiol decrease rapidly whereas the 1evels of corticosterone, thyroxin and tri-iodothryronine are elevated (Hoshino et al., 1988a). Elevated levels of thyroid hormones during post-nuptial molting of non-domesticated and hen during remolding of feathers were observed (Brake et al., 1979; Lien and Siopes, 1989; Etches, 1996). Park et al. (2004d) noted changes in the circulating population of leucocytes during molting. Increased heterophil:lymphocyte ratios were seen in molted hens (Davis et al., 2000; Medvedev et al., 2002; Oguike et al., 2005a).

This rejuvenation increases tissue sensitivity or reproductive efficiency and reorganization of metabolic processes. So regression and redevelopment of organs and tissues are related to the increased reproductive performance post-molt. The decrease in body weight of hens by feed withdrawal is directly related to decrease in muscle, adipose tissue, liver and the involution of reproductive organs (Brake and Thaxton, 1979a; Berry and Brake, 1985; Park et al., 2004a)

The mechanism of renewal of feather in natural molting can be expressed when ovary became atretic which influence in the decreased release of estrogen which suppresses activation of existing feather papillae (Peczely, 1992). Thyroxin/progesterone activate the feather papillae to form an underlying new feather that ultimately expelled its predecessor. Elevated levels of corticosteroids are reported to cause cessation of egg laying as well as gonadal atrophy (Van Tienhoven, 1981; Yousaf and Chaudhry, 2008).

The mechanism of molt induction by the use of dietary zinc is not completely known. However, it has been reported that dietary zinc at high concentrations (10,000–20,000ppm) induce follicular atresia and cessation of laying egg by interfering with ovulation in adult chicken because Zn2+ reduces feed intake to 10–15% of the normal level. But dietary zinc at moderate concentrations (2800ppm) in the absence of a

calcium-supplemented diet has a direct suppressive effect on the reproductive organs because calcium is required for the initiation and stimulation of gonadotropin-releasing hormone which stimulated luteinizing hormone (LH) release (Luck and Scanes, 1980). Zinc–calcium antagonism can also occur and may explain some of the effects observed when dietary zinc is supplemented as a dietary molt-induction compound. Dietary zinc at high concentrations can reduce calcium utilization and dietary calcium is the first limiting mineral for ovulation during the induced molt (Garlich and Parkhurst, 1982). Hens treated with high concentrations of dietary zinc have low plasma progesterone levels and the sensitivity of progesterone to LH is reduced as compared to fasted hens or hens treated with a low-calcium diet (Walker and Frawley, 1977). Zinc causes calcium to fall below a critical level essential for gonadotropin production and release (Berry and Brake, 1985). The effectiveness of zinc is not related to feed consumption and body weight difference, and zinc inhibits production of luteinizing hormone (LH)-stimulated progesterone and the formation of cyclic adenosine monophosphate (cAMP) in granulosa cells of the hen's ovary (Breeding et al., 1992). This inhibition is not caused by a toxic effect of zinc on granulosa cells of the hen ovary. The LH-stimulated progesterone in granulosa cells is dependent on the formation of cAMP and the mobilization of extracellular and intracellular calcium. Extracellular calcium is necessary for gonadotrophin-stimulated cAMP formation (Asem et al., 1987; Park et al., 2004d).

METHODS TO INDUCE MOLT IN BIRDS

Several procedures have been used to initiate molting. These include feed withdrawal (Christmas et al., 1985), water withdrawal (North and Bell, 1999; Ruszler, 1996), **photoperiod** reduction (Akram et al., 2002), feeding low calcium (Breeding et al., 1992) or low sodium diets (Ross and Herrick, 1981; Berry and Brake, 1985; Scheideler et al., 2002), and feeding high dietary zinc (Berry and Brake, 1987; McCormick and Cunningham, 1987; Breeding et al., 1992; Alodan and Mashaly, 1999; Sandhu et al., 2006; Sandhu et al., 2008). Each method can be used alone or in combination with other methods. (Alodan and Mashaly, 1999). Low-sodium diets and high-Zn diets methods have been researched extensively. (Biggs et al., 2004).

The most widely practiced method of molt induction is fasting, however, high levels of dietary zinc oxide supplementation as a molting agent are also used (Alodan and Mashaly, 1999; Sandhu et al., 2006; Yousaf et al., 2008). These alternatives techniques fall into two general categories:

1. Long-term feed withdrawal
2. The use of dietary additives.

The three main methods of forced molting are

(1) Elimination or limitation of food and/or water

(2) Feeding the birds low nutrient rations.

(3) Administration of drugs and metals.

The research reported to date has been inadequate to accurately determine which methods of induced molting are the least stressful compared to the processes experienced by the hen during a natural molt. The three or four most highly refined methods being used commercially are not generally detrimental to the health and welfare of today's laying hen. (Landers et al., 2008a; Ruszler, 1998)

Although many methods are available to induce molting to synchronize the second lay cycle for greater production efficiency, the most widely commercial method is feed withdrawal (Landers et al., 2008b). This method required a longer period for cessation of lay and sometimes resulted in less than optimal rate of lay upon returning to production. The use of chemical or nutritional imbalances to induce molt are usually neither practical nor cost effective because they usually result in poorer postmolt performance. Induced molting is only a management tool after nature that is used to help manage and synchronize the production cycles of a flock. The lack of a common optimal program arises from differences in genetics, age, environment, and previous flock production history in the various studies reported. It should be obvious that which methods caused unnecessary stress and are not humane. (Ruszler, 1998)

However, although during natural molting feed intake, activity, and body weight are reduced, the method of extended feed withdrawal is considered harmful to bird welfare. Therefore, there is an increasing interest in the research of alternative methods that reduce stress and present the same economic results as the conventional method applied in forced molting (Ramos et al., 1999; Faitarone et al., 2008b).

FEED OR NUTRIENT RESTRICTION

Feed withdrawal is the primary procedure to induce molting and stimulate multiple egg laying cycles in hens (Bell, 2003; Biggs et al., 2004; Chowdhury et al., 2004; El-Deek and Al-Harthi, 2004; Oguike et al., 2004; Kubena et al., 2005; Khoshoei and Khajali, 2006; Venkata et al., 2008). Excessive body weight loss, lowered immunity and higher **mortality are seen** in feed withdrawal methods, while laying hens molted by non-feed withdrawal exhibit lower body weight loss, better immunity, less mortality and sequential rejuvenation of the reproductive system. (Yousaf and Chaudhry, 2008).

ADVANTAGE OF ZINC OVER FEED RESTRICTION METHOD

Birds molted by feed withdrawal may be more susceptible to S. enteritidis colonization and invasion. Therefore, in recent years, attempts have been made to devise dietary approaches that optimize molt induction but retain gastrointestinal tract function, indigenous gastrointestinal microflora, and bird physiology (Scott and Creger, 1976; Gast and Ricke, 2003). Given the success of zinc-amended diets for molt induction, these diets have been suggested as a means to limit S. enteritidis colonization and organ invasion as well. Therefore, zinc-molting diets were recently investigated to evaluate whether zinc feeding might have an effect of induced molt and influence the hen's S. enteritidis infection. Low-calcium (800ppm Ca), low calcium–low zinc (800ppm Ca/110ppm Zn) (Ricke et al., 2004) and high zinc (10,000ppm) zinc-containing molting diets (Moore et al., 2004) decreased S. enteritidis colonization in laying hens compared to feed withdrawal. In a study involving different zinc organic salts (Moore et al., 2004), zinc acetate (1% Zn) appeared to be more effective for inducing molt and stimulating multiple laying cycles without increasing the risk of S. enteritidis, whereas zinc propionate (1% Zn) feeding was consumed in smaller quantities and was generally less effective for reducing the risk of S. enteritidis contamination. Lower feed consumption might also lower the production of lactic and/or volatile fatty acid in crop and caeca of the molted hens by the indigenous microflora (Moore et al., 2004). This fact was in accordance with Ricke et al. (2004) who reported that feeding low calcium and zinc molt diets retain sufficient fermentative activity to limit S. enteritidis colonization

and, therefore, were generally more effective in preventing extensive *S. enteritidis* invasion (Park et al., 2004e).

The use of various levels of dietary zinc (as zinc oxide) for inducing pauses in egg production had reported by several researchers (McCormick and Cunningham, 1984a and 1987; Berry and Brake, 1987; Breeding et al., 1992; Bell, 2003; El-Gendi et al., 2009)

DIETARY METALS (FEED ADDITIVES)

Dietary zinc is effective at inducing rests from egg production, with post rest production performance being similar to that of induced molt programs involving feed withdrawal (Cunningham and McCormick, 1985; Goodman et al., 1986; Berry and Brake, 1987). Zinc has an inhibitory effect on ovarian function (Johnson and Brake, 1992) and when incorporated at relatively low levels in low calcium diets it causes oviposition to cease without greatly depressing feed consumption (Breeding et al., 1992). Increasing levels of dietary zinc, however, cause progressive declines in feed consumption and hens given 20,000ppm zinc in an otherwise typical layer ration virtually begin fasting (McCormick and Cunningham, 1984a, 1987). Dietary aluminum has also been tried as a molting agent (Hussein et al., 1989). The procedure can take more than 2-wk to cause egg production to cease, but production performance during a 14-wk second cycle comparison was similar to that of hens molted by feed withdrawal. Dietary aluminum also causes reduced feed intake by hens.

Holt et al. (1994) reported that laying hens on alternative molt diets excrete less S. enteritidis in their excreta, showed less susceptibility to an S. enteritidis infection and less intestinal inflammation as compared to fasted hens, although the percentage of S. enteritidis- positive birds did not differ between molted groups. Recently, Seo et al. (2001) found that feeding wheat middlings, by-products of wheat flour that contain low fibre and higher energy, resulted in cessation of egg production within 7 days, with no increased risk for S. enteritidis. Alfalfa molt diets have also been shown to limit S. enteritidis infection of laying hens (Kwon et al., 2001). High zinc (10,000 **mg/kg**) as Zn acetate 10,000 (Moore et al., 2004) and low calcium (80mg/kg) or low calcium (80mg/kg)-moderate zinc (110 mg/kg) (Ricke, 2003) were very effective for

inducing molt and stimulating multiple laying cycles without increasing the risk of S. enteritidis. These molt diets not only appear to retain sufficient protective microflora during induced molting but support sufficient fermentative activity to limit S. enteritidis colonization to the same degree as birds on a non-molt layer diet. Availability of such diets would avoid the more drastic influence on the laying hen's gastrointestinal tract microenvironment that feed withdrawal incurs and the subsequent increases in S. enteritidis colonization and infection (Durant et al., 1999; Ricke, 2003; Park et al., 2004e)

Inductions of molt through dietary mineral additives such as Cu, Zn, Na and Al have been practiced by various scientists to halt egg production and enhance the post-molt production (Stevenson and Jackson, 1984; Harms, 1981; Hussein et al., 1989). High levels of either aluminium salt (Hussein et al., 1988; Hussein, 1996; Yousaf, 2004; Yousaf and Ahmad, 2006) or dietary zinc (Yousaf et al., 1998; Yousaf, 1998; ElDeek and Al-Harthi, 2004; Moore et al., 2004; Ocak et al., 2004; Ahmed et al., 2005; Yousaf and Ahmad, 2006; Koch et al., 2007; Koelkebeck and Anderson, 2007; Yousaf and Chaudhry, 2008) or potassium iodide (McCormick and Cunningham, 1987; Hussein et al., 1989) can be used in alternative methods of molting. Yousaf and Chaudhry (2008) reported additional research that the use of various additives such as progesterone and enheptin can be helpful in inducing molting.

However, induced molting by high dietary minerals has raised public health concerns regarding the potential deposition of these minerals in eggs and meat, which may affect human health. The risk of high mineral residues can be reduced in egg laying hens by using low mineral diets. (Yousaf and Chaudhry, 2008)

Research on alternative molting programs at the University of Nebraska revolved around the feeding of "nutrient-balanced" diets (1,250 kcal of MEn/lb, 10 and 12.5% protein, 1.5% calcium, and 0.5% available phosphorus) with 0% added salt compared with a conventional feed withdrawal program (Scheideler et al., 2003). Their program also called for increasing the photoperiod to 16 or 24h of light for one week before the initiation of the molt treatments. In their research, the level of sodium did not affect feed intake; however, cessation of lay and body weight loss were not as complete as in hens molted by an 8 to 10 day fasting method. In addition, they found that fasted birds had better egg shell quality in the postmolt production period. (Koelkebeck et

al., 2006). Relative performance of flocks molted by traditional feed removal methods was evaluated compared with flocks fed diets with low levels of sodium, calcium, and protein. In general, egg production and body weight losses differed between the two molting methods during the first four weeks of the test, but performance after that was similar (Koelkebeck et al., 2006).

To minimize the potential bird stress, various methods of nutrient restriction that would avoid long term feed withdrawal have been investigated (Berry, 2003; Webster, 2003; Park et al., 2004c). Alternative methods for molt induction include feeding low-Ca or low-Na diets and high-dietary Zn or high-fiber, low-energy diets (Berry and Brake, 1985; Breeding et al., 1992; Seo et al., 2001; Biggs et al., 2003, 2004; Donalson et al., 2005; Landers et al., 2005a; Woodward et al., 2005). Each method can typically be applied in combination with a change in the photoperiod and usually causes body weight loss, regression of the reproductive system, cessation of egg production and induction of a molt (Bell, 2003; Park et al., 2004b). Among the high-fiber, low-energy molt diets, alfalfa molt diets effectively regresses the reproductive system and bring about a rapid return to egg lay at a rate similar to feed-withdrawal hens (Donalson et al., 2005; Landers et al., 2005b). Alfalfa diets also lower *Salmonella Enteritidis* colonization in the organs and reduce intestinal shedding of the pathogen during molting compared to hens subjected to feed-withdrawal (McReynolds et al., 2005, 2006; Woodward et al., 2005; Dunkley et al., 2007, 2008a).

HIGH ZINC MOLTS

Hens that received a Zn molting ration for seven day resumed egg production one week after returning to a normal diet. Hens that received a Zn ration for 12 days resumed production 25 days after removal of excess Zn from the diet (Scott and Creger, 1976). Shippee et al. (1979) found that hens molted with a Zn ration returned to production two week after the end of treatment and reached 50% production by five weeks after the initiation of the molt. Arrington et al. (1967) found that of the hens molted by use of a 28 day high I (Iodine) diet, 85% returned to production within ten days after removal of excess I (Iodine) from the feed. The remaining 15% of the flock did not resume production (Berry, 2003).

High concentrations of zinc added to poultry layer ration have been experimentally used as an alternative method to induce molt. Zinc at 20,000ppm added to the diet was effective in inducing molt and generally gave results comparable to, if not significantly better than, those obtained with feed removal (Creger and Scott, 1977; Roberson and Francis, 1979). The 20,000ppm (2%) of zinc as zinc oxide caused a complete stop of egg production within five days and resulted in significant improvements of production in the periods of postmolt compared with that observed immediately premolt (Creger and Scott, 1977). The addition of 10,000ppm (1%) zinc as either zinc oxide or zinc acetate to the layer ration for 14 day caused egg production to decline from 60% to 0% within 6 days (Shippee et al., 1979). Ten thousand parts per million (1%) of zinc given as zinc propionate in the supplemented diet caused a complete induced molt and egg weights from Zn propionate fed hens were heavier than those from feed-withdrawal treatment hens (Park et al., 2004a). Hens fed high concentrations of zinc ceased ovulating a day sooner than hens molted by feed withdrawal (Park et al., 2004a; Berry and Brake, 1985). There were no differences in the reproductive systems between hens molted by feed withdrawal and hens molted by high dietary zinc (Park et al., 2004c; McCormick and Cunningham, 1987). These authors also indicated that the effectiveness of zinc at high concentrations might be the result of depression of feed intake. However, moderate concentrations of zinc (2800ppm) were effective for suppression of hen reproduction systems (Park et al., 2004b).

Induction of molting through high dietary mineral zinc (Cantor and Johnson, 1984; Yousaf et al., 1998; El-Deek and Al-Harthi, 2004; Moore et al., 2004; Ocak et al., 2004; Ahmed et al., 2005; Yousaf and Ahmad, 2006; Koch et al., 2007; Koelkebeck and Anderson, 2007; Reddy, et al., 2008; Venkata et al., 2008) has been successfully used.

A high-zinc diet has received more attention as a potential molting diet since addition of a trace component is much easier to execute in practical molting settings than to reproduce a well balanced low calcium and sodium diet (Ruszler, 1998). A diet with 10,000 to 25,000ppm given as zinc oxide or zinc acetate resulted in cessation of egg production within 5 to 7 days (Shippee et al., 1979; Park et al., 2004a).

High zinc diets produced results comparable to feed withdrawal among all alternative methods but there is incomplete regression of reproductive tissue with this method that may affect the postmolt shell quality leading to increased losses. Improvement in the existing alternative methods or development of a new feasible alternative will help to make commercial poultry more profitable. A better understanding of reproductive tissue remodeling mechanism during molting is a step forward in this direction (Anish et al., 2008).

Previous studies conducted on non withdrawal feeding programs have utilized various techniques to induce a molt. Among them feeding of high levels of zinc is used as alternative feed ingredient. In one study, laying hens were fed very high levels of zinc and it was found that egg production was slightly better and egg weights were heavier for hens induced to molt when fed 1% zinc acetate and 1% zinc propionate compared with a conventional feed withdrawal method (Park et al., 2004b). Using cottonseed diets, researchers found that feeding hens a diet containing 50% finely ground cottonseed produced voluntary feed intake reduction. This method was determined to be equivalent in effectiveness to a complete feed withdrawal program (Davis et al., 2002). In another study, the feeding of a diet composed mostly of grape pomace containing 10ppm of thyroxin was effective in supporting similar postmolt performance as a conventional feed withdrawal method (Keshavarz and Quimby, 2002). Thus, the above-mentioned studies and many others have documented that laying hens can be molted by other means than a conventional feed withdrawal (Koelkebeck et al., 2006)

Zinc, a natural trace mineral in feed, is cheaper and can be added easily to feed making this molting program more practically feasible compared with other alternative methods (Ruszler, 1998). Molting by zinc reduces the risk of Salmonella enterica serovar enteritidis colonization in hens and its vertical transmission through eggs (Moore et al., 2004; Park et al., 2004e). A high dose of zinc (10,000–20,000ppm) causes a cessation of ovulation a day sooner than feed withdrawal (Park et al., 2004d). In most studies, dietary zinc at high concentrations has been reported to induce follicular atresia and cessation of egg laying by reducing feed intake to 10%–15% from normal (Breeding et al., 1992). Further, zinc has a direct suppressive effect on reproductive organs by means of inhibiting the utilization of calcium (Garlich and

Parkhurst, 1982; Berry and Brake, 1985), which is required for the gonoadotropin secretion (Simkiss, 1961; Taylor, 1965). High doses of zinc have also been found to inhibit the production of luteinizing-hormone induced progesterone production and the formation of cAMP in the granulosa cells of the ovary (Luck and Scanes, 1980 and Sundaresan et al., 2008b)

Dietary zinc is effective at inducing rests from egg production, with postrest production performance being similar to that of induced molt programs involving feed withdrawal (Shippee et al., 1979; Cunningham and McCormick, 1985; Goodman et al., 1986; Berry and Brake, 1987). Zinc has an inhibitory effect on ovarian function (Johnson and Brake, 1992) and when incorporated at relatively low levels in low calcium diets it causes oviposition to cease without greatly depressing feed consumption (Breeding et al., 1992). Increasing levels of dietary zinc however, cause progressive declines in feed consumption such that hens given 20,000ppm zinc in an otherwise typical layer ration virtually begin fasting (McCormick and Cunningham, 1984, 1987 and Webster, 2003).

Using high level of dietary zinc is an effective method of inducing rest for laying hens (Scott and Creger, 1997; Berry and Brake, 1987). Force molting of layers is used as a management technique to avoid the annual cost of replacing pullets. Force molting, which is characterized by cessation of egg production for several weeks, may improve rate of production during postusing zinc oxide (McCormick and Cunningham, 1984b; Berry and Brake, 1987; Mohamed, 1987; Mohamed, 1990; Hassan, 1996; El-Deek and Al-Harthi 2004)

LOW CALCIUM MOLTS

Calcium is an important element for maintenance of egg production in the chicken, not only because large amounts of it are necessary for shell formation, but also because it plays a role in the secretion of gonadotrophic hormones (Decuypere and Verheyen, 1986; Brake, 1993). Attempts have been made to arrest egg production of hens by feeding diets low in calcium (Rolon et al., 1993). These were effective at reducing egg production, with the diets lowest in calcium causing the lowest rates of production, but did not cause flocks to cease production entirely. Even at very low levels of dietary calcium, the ovary and oviduct did not regress to a non-reproductive

state (Douglas et al., 1972; Gilbert and Blair, 1975). Peak egg production tends not to be as high after rests induced by low-calcium diets as after rests induced by feed deprivation, but some improvement in shell quality can be achieved (Hurwitz et al., 1975; Rolon et al., 1993). A problem regarding diets very low in calcium is that temporary paralysis (Douglas et al., 1972) or osteoporosis (Hurwitz et al., 1975) can occur. Hughes and Wood-Gush (1973) observed that calcium deprivation causes increased activity and pecking in pullets. Diets deficient in sodium have been used to induce molt (Berry and Brake, 1987). This method may not cause hens to completely cease egg production and gives inferior second cycle performance. Hughes and Whitehead (1979) reported increased cannibalistic pecking among hens fed low sodium diets.

LOW SODIUM MOLTS

A low-sodium diet less than 40ppm reduced the rate of egg production to less than 5% within 14 to 21 days and in some cases resulted in a complete cessation of egg laying within 4 weeks. (Park et al., 2004a) The use of a low sodium diet (Naber et al., 1984; Said et al., 1984) is equally effective as a feed restriction technique for inducing molt. Copper is used as an effective molting agent (Pearce et al., 1983; Stevenson and Jackson, 1984; Yousaf, 1998; Yousaf, 2004). Nutrient restrictions have been successfully employed using marginal levels of salt or sodium (Naber et al., 1984), calcium (Martin et al., 1973) and low-nutrient diets (Bell et al., 1976; Yousaf, 1998; Yousaf and Chaudhry, 2008)

ENHEPTIN

The drug Enheptin when fed in the diet can induce molt in hens. Dietary enheptin reduces feed consumption of hens and appears to cause dose-related body weight loss (Webster, 2003).

DIETARY IODINE

High levels of dietary iodine have been shown to induce molt in hens (Wilson et al., 1967). Second-cycle production performance was inferior to the unmolted control in this study (Webster, 2003).

HORMONE AGONIST AND HORMONES

The reproductive hormone, progesterone, when injected or fed in the diet will cause late-cycle hens to go out of production (Adams, 1955, 1956; Wilson et al., 1967). When progesterone is injected, the process is fast, but when the hormone is fed it tends to be slow to take effect. Improvement in egg production appears to be possible, but the use of progesterone as a molting agent for commercial use would require further study in a modern scenario. The thyroid hormone (thyroxin) has been shown to terminate egg production and induce molting of hens (Sekimoto et al., 1987).

LOW ENERGY DIET

An alternative dietary approach to feed withdrawal molt induction has been to implement a low energy complete diet that can be fed ad libitum. Although diets varying in nutrient density have been used successfully in molting birds in different situations, it is difficult to pinpoint a single nutrient or feed type for this purpose (Yousaf and Chaudhry, 2008).

EGG PRODUCTION

Lee (1982) reported that molted birds exhibit greater egg production, better feed efficiency, better shell quality, and less mortality than unmolted birds. A greater primary feather loss is associated with greater egg production (Lee, 1982). The peaks of egg production during the second cycle are approximately 75 to 85% (Bell, 2003). One of the main reasons for increased post-molt egg production is decreased post-molt production of shell-less eggs (Roland and Brake, 1982). Hens that lay shell-less or poorly shelled eggs show increased shell gland lipid (Roland et al., 1977). This lipid is largely confined to the calcium secreting glandular epithelium (Baker et al., 1983) and remains during the feed withdrawal periods until more than 25% of the bird's initial weight is lost (Brake, 1992; Park et al., 2004a).

Circulating plasma concentrations of progesterone, LH, and estradiol are lower in molting laying hens than in unmolting laying hens while corticosterone, thyroxin (T_4) and tri-iodothyronine (T_3) levels increase during the molt (Hoshino et al., 1988b). Hoshino et al. (1988a) indicated that the declines of LH, estradiol, and progesterone were coincident with the cessation of egg production (Park et al., 2004a)

It is well-known that induced molting improves eggshell quality and egg production (Berry and Brake, 1991; Brake, 1993; Hurwitz et al., 1998). Heryanto et al. (1997a) suggests that the regression and removal of the old tissues and the recovery of the tissues with proliferation and cytodifferentiation of new cells may improve post-molting egg production and egg quality. In particular, rejuvenation of shell gland tissue may increase calcium-binding protein (Calbindin), resulting in improvement of egg shell quality (Heryanto et al., 1997b). Calbindin increases with the onset of egg production and decreases as egg production reduces (Nys et al., 1989). The relationship between egg shell thickness and the shell gland calbindin is positively correlated (Nys et al., 1986; Park et al., 2004a)

Alodan and Mashaly (1999) demonstrated that induced molting significantly increased egg production from 64% to 77 to 83%, Haugh units from 80.4 to 85.9 to 87.3, and shell weight from 5.3g to 6.3 to 6.4g when compared to control. The body weight of the molted hens decreased significantly to 84.8, 74.5, and 88% of the initial body weight for Zn, CAL, and on-off groups, respectively. The total number of circulating leukocytes was significantly lower in molted hens than in control hens. (Alodan and Mashaly, 1999)

Postmolt performance of the laying hens includes egg size, shell quality, internal egg quality, and the rate of egg production. Egg size is increased significantly after a molt with a higher percentage of eggs graded large (Zeelen, 1975). Shell weight of eggs of the molted hens are also improved (Zimmermann et al., 1987). In addition, there is a marked increase in the interior egg quality, as measured by Haugh units, following molting (Zimmermann et al., 1987). The most important improvement is the increase in the rate of egg production during the postmolting period (Christmas et al., 1985; Wilson et al., 1967). Because induced molting includes dietary restriction, it can affect the different components of the immune system such as thymus (Brake et al., 1981ab) and spleen (Brake et al., 1985) weights. Furthermore, Ben Nathan et al (1977), Brake et al (1982), Holt (1992b), Holt and Porter (1992b) reported that the total number of circulating leukocytes decreased significantly during molting, especially during the early stages. Furthermore, molting has an effect on differential white blood cell counts mainly by increasing the heterophils and decreasing the lymphocyte cell number in peripheral blood (Wolford and Ringer, 1962). In addition,

molting also caused a reduction in the total number of circulating lymphocytes (Holt, 1992a; Holt and Porter, 1992a and Alodan and Mashaly, 1999).

Both feed removal and the wheat middlings treatments resulted in total cessation of egg production within 8 days. Egg production of hens fed the corn molt diet had decreased to 3% by 28th day. Body weight loss of hens fed the corn or wheat middlings diet was approximately 15 and 8% at 28th day respectively (Biggs et al., 2003). Egg production during molt decreased to <5% by week 2 and remained at that level for approximately 3 weeks. The highest egg production in a single week was reached in 8th week of the first cycle (89.4%), in 13th week in the second cycle (79.5%), and in 13th week in the third cycle (74.7%) (Bell, 2003). Molting induced physiological stress resulted in regression of the ovarian tissues. This in turn led to reduction of ovarian activities with decrease in progesterone production. However, the regeneration of large yellow follicles and secretion of progesterone commenced after molt induction period. These changes indicated the rejuvenation processes and the reactivation of ovarian tissues associated with forced-molt (Oguike et al., 2005b).

The most effective method that will generate optimal egg production for the longest period has been one using a period of feed withdrawal. Most commercial egg producers use molting programs that have some period of feed withdrawal because they are effective and can be easily followed. A bird going through a natural molt will reject feed for an extended period of time. There is concern centering upon whether it is harmful to initiate a molt before the bird is physiologically ready (Mrosovsky and Sherry, 1980). This concern, plus the desire for a simple method to molt hens, has led to extensive research into methods that do not require feed deprivation. Producers and researchers are encouraged to work together to develop alternatives to feed withdrawal for molting. Until such time that these alternatives are available, the shortest period of feed withdrawal possible should be used to accomplish the goal of rejuvenating the hen's egg production capabilities and overall welfare. Ruszler and Minear (1997) noted that a low-energy and high-fiber molt diet full fed to hens after a 4-day feed withdrawal induced molt that was equally effective as other commercial molts using longer periods of feed withdrawal. It was reported that low-energy and high-fiber diets fed to commercial flocks with no period of feed withdrawal would induce a molt **(**Minear, 1999; Ruszler and Novak 2006).

During forced molting, cessation of egg production occurs with ovarian atrophy (Brake and Thaxton, 1979a; Berry and Brake, 1985; Verheyen, 1986). Follicular atresia is the result of a reduced LH stimulation due to the refractoriness of the hypothalamus and probably also the pituitary to progesterone stimulation during fasting (Tanaka et al., 1974; Johnson and Van Tienhoven, 1980a; Verheyen et al., 1987). Egg production is at a relatively low level towards the end of laying cycle when interior and exterior egg quality are also poor, particularly if the tail end coincides with hot weather (Yousaf and Ahmad, 2006). It is assumed that induced molting exerts its effect on egg producing functions by rejuvenation of reproductive organs, including regression and remodeling of oviductal tissues (Heryanto et al., 1997a). High concentration of Zn additive significantly reduced food, water, egg production and body weight. Zinc acetate having a greater detrimental effect than the oxide at equivalent amount added as Zn inclusion. Zinc acetate depressed live body weight, weight of the kidneys, ovary and oviduct to a greater extent than the oxide treatments (Gibson et al., 1986). A pullet (young female chicken) begins laying eggs at 18 to 20 weeks of age. She reaches peak production at about 35 weeks, with a production rate greater than 90 percent. The average commercial Single Comb White Leghorn hen lays about 265 eggs per year, with backyard breeds laying fewer (Hermes, 2003b).

HORMONAL PROFILE DURING MOLTING

During the process of molting, levels of corticosteroids increase significantly and concentrations of estrogen, progesterone and luteinizing hormone (LH) decrease sharply (Sandhu et al., 2008). These findings suggest that the pituitary gland plays a central role in the control of the many endocrine functions which are altered during molting. Sandhu et al (2008; 2010) studied the variations of different cells in the pituitary gland of laying hens in response to Zn- induced molting through immuno-histochemical techniques and found that LH gonadotroph cell size was significantly higher in Zn-induced molted birds compared to those treated with the fasting method. Plasma Zn levels increase after Zn-induced molting which may result increase in LH gonadotrophs and nucleus size because of their greater accumulation in mRNA or ribosome's (Sandhu et al., 2006). Gonadotrophic-releasing hormone stimulates the

production of the gonadotropins from the anterior pituitary gland, thereby raising the plasma levels of the gonadotropins. Increasing levels of luteinizing hormone (LH) in the plasma perfusing the largest ovarian follicle stimulates further secretion of progesterone and the rising progesterone continues to drive LH release. This positive feedback between progesterone and luteinizing hormone triggers LH surge that leads to ovulation of the largest follicle (Etches, 1996). In laying hens, the primary sources of progesterone are the granulosa cells of the five largest follicles in the hierarchy (Verheyen et al., 1987; Etches, 1984). Therefore, progesterone is the only steroid produced by the ovulating follicle (Imai and Nalbandov, 1978; Decuypere et al., 1993). On the other hand, small white follicles are the major sources of estrogens, producing about 87% of the total ovarian estrogens (Senior and Furr, 1975; Gilbert and Wells, 1984). Luteinizing hormone and presumably follicle stimulating hormone, levels decline as corticosterone levels increase at the beginning of the fast. A possible mechanism for this is that corticosterone makes the pituitary refractory to GnRH. In the laying hen, corticosterone levels have an inverse relationship with LH (Wilson and Cunningham, 1980).

Follicle stimulating hormone (FSH) plays vital roles in the control and regulation of follicular recruitment, growth and maintenance of large yolk filled follicles. The number of growing follicles in the ovary and the incorporation of yolk into small follicles are stimulated by FSH without disrupting the hierarchy (Mitchell, 1970). Plasma levels of vitellogenin were observed to fluctuate greatly during ovarian cycle with the highest level coinciding with the zenith of estradiol (Callard et al., 1978; Ho et al., 1982; Lance, 1989 and Carnevali et al., 1991). FSH showed significant increase of 50% during the period of feed restriction. FSH was reported to increase from 2.14±0.11 to 3.19±0.20 ng/ml (Vanmontfort et al., 1994). These authors also reported that LH showed slight but significant overall decrease from 2.77±0.23 to 2.14±0.21ng/ml after ovarian regression following starvation. Furthermore, progesterone was significantly negatively correlated with FSH and significantly positively correlated with LH during starvation (Vanmontfort et al., 1994). Redevelopment of follicular sized hierarchy started after resumption of feeding a layer mash. Conditions of stress have been observed to affect vitellogenin production adversely. These authors also observed positive significant correlation between

follicular atresia and decline in serum vitellin following fasting of laying hens. A main function for follicle-stimulating hormone (FSH) is closely related to granulosa cell differentiation and the induction of steroidogenesis in prehierarchal follicle granulosa cells (Etches, 1990). FSH stimulates both cyclic adenosine monophosphate (CAMP) formation and progesterone secretion by the granulosa of intermediate stage follicles.

Prior to first oviposition, plasma level of progesterone was found to rise and remain within the range of 0.4 to 2.2ng/ml while the birds were in lay (Williams and Sharp, 1977). The highest plasma levels of progesterone and LH are between 4 to 7 hours before ovulation (Wilson and Sharp, 1973; Silverside et al., 1993). The reduced rate of follicular growth in aging hens may be as a result of inadequate stimulation of the ovary by FSH. Joyner et al (1987) recorded plasma progesterone levels of 4.29, 3.80 and 1.64ng/ml for pullets, old layers and old non-laying hens, respectively. They also recorded respective estradiol levels for these birds as 184.9, 181.7 and 66.7pg/ml.

During molting, plasma levels of LH, progesterone and estradiol decline rapidly whereas the 1evels of corticosterone, thyroxin and tri-iodothryronine are elevated (Hoshino et al., 1988ab). During induced molting, the large follicles were reabsorbed and the regressed ovary was left with many small white follicles (Oguike, 2004; Su et al., 1995). Follicular atresia was reported to be a result of reduced LH stimulation due to refractoriness of the hypothalamus and probably also the hypophysis to progesterone stimulation during fasting (Johnson and Van Tienhoven, 1980b). Furthermore, Etches (1996) observed that involution of the reproductive tract appears to be initiated by the hypothalamo-pituitary axis since the pituitary gland becomes refractive to stimulation of exogenous GnRH. Starvation prevented egg laying and significantly reduced plasma levels of sex steroids. Tanabe et al (1981) reported no change in pituitary LH during and following starvation but Vanmontfort et al (1994) noted significant overall decrease after ovarian regression following starvation. These authors also reported plasma levels of progesterone and estradiol ranged between 0.51-0.72ng/ml and 58.9 and 101.0pg/ml during starvation and 1.38ng/ml and 173.5pg/ml when fed ad libitum. Progesterone was significantly negatively correlated with FSH and significantly positively correlated with LH during starvation (Vanmontfort et al., 1994). Tanabe et al (1981) reported decreased plasma levels of

progesterone and estradiol in laying hens during starvation. They also observed that plasma progesterone levels ranged between 0.51 and 0.72ng/ml during starvation, in contrast with 1.38ng/ml when fed ad libitum. Resumption of feeding following starvation raised plasma levels of LH and progesterone and estradiol to two folds of its initial levels within 3 to 4 days of re-feeding. Thereafter, the levels of estrogen declined to its normal concentration. Redevelopment of follicular sized hierarchy started after resumption of feeding a layer mash.

OVARIAN FUNCTIONS DURING MOLTING

Ovarian regression begins soon after the start of fasting with declines in estradiol and progesterone (Tanabe et al., 1981; Etches et al., 1984). As LH and follicle stimulating hormone levels rise following refeeding and photo stimulation, ovarian follicles enter into the maturational hierarchy and begin producing estradiol and progesterone. Refeeding of hens and exposure to long day lengths following induction of molt, hypothalamic GnRH release is resumed and pituitary responsiveness is restored. This causes increase in LH concentration, which stimulates ovarian development and ovarian steroid production. Estrogen and progesterone levels increase. Corticosterone levels decline, but may show a transient increase as egg production resumes. Ovarian function of the hen depends on the information relayed from the hypothalamo-hypophyseal axis as well as from the ovarian tissues themselves. Natural molt occurs with advancement in the age and length of intensive production of a laying flock. Etches (1996) indicated that molting occurs when plasma concentrations of steroids and gonadotropins are low. This is in agreement with the findings of Decuypere and Verheyen (1986) and Jacquet et al. (1993) who reported that changes in the reproductive functions during forced-molting were associated with reduced levels of luteinizing hormone and sex steroids. The major changes caused by molting within the hypothalamo-hypophyseal-gonadal axes result in temporary cessation of lay.

Etches (1996) reported that within 14 days of molt induction, the large follicles were reabsorbed and the regressed ovary was left with many small follicles. Su et al. (1995) reported increased number of large white follicles in the ovaries of albino and non-albino hens following forced-molt. Follicular atresia was reported to be partly due to refractoriness of the hypothalamus and probably also the hypophysis to progesterone

stimulation during fasting (Johnson and Van Tienhoven, 1980b and Oguike et al., 2005a). Androgens that are produced by the thecal cells of the second and third largest follicles are aromatized to estrogens by these follicles (Huang and Nalbandov, 1978; Armstrong, 1985). The production of estrogens and androgens decreases in the hierarchical follicles while that of progesterone is increased in the granulosa cells of these follicles. Therefore, progesterone is the only steroid produced by the ovulating follicle (Imai and Nalbandov, 1978; Etches and Duke, 1984; Decuypere et al., 1993).
In laying hens, the primary sources of progesterone are the granulosa cells of the five largest follicles in the hierarchy of the ovary. Progesterone produced by the follicle makes the major contribution to the preovulatory peak of progesterone (Hammond et al., 1981; Etches, 1984; Wells et al., 1985). However, some authors argue that the small white follicles (<5mm in diameter) of the ovary are the major source of estrogens since they are responsible for about 87% of the total ovarian content (Gilbert and Wells, 1984; Armstrong, 1985). Production and secretion of progesterone are mainly controlled by luteinizing hormone (LH) (Hammond et al., 1981). The regulation of estradiol secretion by the thecal cells is less clear (Bahr et al., 1980).

CORTICOSTERONE.

The first significant endocrine change observed in fasted hens is that corticosterone levels increase as the hypothalamic-pituitary-adrenal (HPA) axis is activated by the need to mobilize body energy stores. The circulating levels of adrenal cortical hormones decreased during molting when photoperiod is reduced (Gildersleeve et al., 1979). Etches et al. (1983) reported that corticosterone is elevated in hens during molting induced by fasting. The degree of the increase in corticosterone depends on the method used to induce molt. Methods such as fasting, which induce a rapid and complete molt, are associated with larger increases in corticosterone levels (Etches et al., 1983). In the fasting hen, increased corticosterone levels are associated with inhibition of the HPG axis (Williams et al., 1985). Corticosterone controls the timing of the preovulatory LH surge (Wilson and Cunningham, 1980) but there is no ovulation-related increase in circulating corticosterone. However, the interaction of the HPA axis and the HPG axis is complex. In the hen, corticosterone may either augment or inhibit reproductive function depending on the season, environmental

circumstances, and availability of feed (Carsia and Harvey, 2000). In the case of zinc-induced reproductive regression, the level of corticosterone is not up regulated, but estrogen and progesterone decrease from basal levels (Braw-Tal et al., 2004). However, serum corticosterone levels are upregulated in the molting induced by feed withdrawal. Therefore, the influence of zinc on corticosterone during reproductive remodeling is unclear. During the period of induced molting, significant increases in circulating levels of corticosterone and thyroid hormone occur (Dickerman et al., 1992) and concentrations of estrogens, progesterone and luteinizing hormone (LH) decrease (Tanabe et al., 1981). Elevation of corticosterone, the major glucocorticoid in birds, leads to a series of events that can enhance short-term survival, including redirected behavior and mobilization of energy reserves (Wingfield et al., 1998). The half-life of these hormones is short (minutes to hours), so their levels drop and their effects disappear if the stressor is removed. In birds the concentration of corticosterone in blood plasma is a reliable indicator of stress (Beuving and Vonder, 1986) and a daily rhythm of corticosterone has been shown to support the concept that the dark phase of the photoperiod triggers the daily rise in plasma corticosterone in diurnally active birds (Joseph and Meier, 1973). Circulating corticosterone is extensively metabolized by the liver with a half-life of 10-15 min in chickens (*Gallus domesticus*) and ducks (Bradley and Holmes, 1971) Secretion of corticosteroids can also be pharmacologically induced by administration of ACTH. In chickens the ACTH stimulation resulted in a dramatic increase of plasma corticosterone levels up to 10-fold (Harvey et al., 1980, 1983).

Feed withdrawal or feed restriction causes increased concentrations of plasma corticosterone in immature chickens (Freeman et al., 1980; Harvey and Klandorf, 1983; Mench, 1991). Freeman et al. (1981) found that plasma corticosterone concentrations were elevated in feed-restricted chickens at first, but that these concentrations declined to equal those of full-fed chickens by 7 week of age. The birds that were more restricted from feed, thus smaller, had higher plasma corticosterone levels at any given age. Hocking et al. (1998) reported that plasma corticosterone was elevated in feed-restricted mature broiler breeder hens. An early period of feed limitation eliminated subsequent plasma corticosterone increase in response to feed withdrawal in growing chickens. Plasma corticosterone appears to be

linked to bird activity in feed-deprivation situations. Hocking et al. (1996) noted that plasma corticosterone levels and activity levels in broiler breeders were higher when the degree of feed restriction was greater. Glucocorticoids stimulate hepatic gluconeogenesis, helping to maintain levels of plasma glucose (Munck et al., 1984). The hyperglycemic effect of corticosterone, the glucocorticoid active in domestic fowl, was demonstrated in White Leghorn hens by Greenman and Zarrow (1961).

Increases in plasma levels of adrenocortical hormone, corticosterone (CS), have been reported to be an indicator of acute stress in poultry (Beuving and Vonder, 1978; Gross and Siegel, 1983; Craig and Craig, 1985; Davis and Siopes, 1985), whereas increases in heterophil to lymphocyte ratios (H:L) caused by heterophilia have been reported to be an indicator of chronic stress (Gross and Siegel, 1983; Gross, 1989; Maxwell, 1993, Maxwell et al., 1992; Siegel, 1995). The process of molting hens in the commercial egg industry can be characterized as a period of considerable physiological change. (Beuving and Vonder, 1978; Hoshino et al., 1988a).

EFFECT OF MOLTING ON ORGAN SYSTEMS

Loss of gonadotropin support during fasting causes involution of the ovary. Follicles in the maturational hierarchy become atretic and the yolk material is reabsorbed. Ovary weight declines as follicles become atretic. Reduction in ovary weight is initially dependent on the duration of fasting and the rate of BW loss. Involution of the oviduct follows the loss of ovarian steroidal support. Regression of the oviduct is a true remodeling of the tissue rather than a decline in the size of cells or shrinkage of the tissue. Apoptosis removes cells of the glandular epithelium during regression (Heryanto et al., 1997b). Remodeling even extends to the connective tissue of the oviduct as evidenced by increased levels of collagenase activity during involution. Upon resumption of sex steroid production by the ovary during recovery from the molt, the oviduct recrudesces. Evidence for changes at the cellular level in the oviduct were found in reports from several researchers. Baker (1981) observed that the uterine glandular epithelium, which is the site of egg shell Ca transport and deposition, contain quantities of intracellular lipid visibly detectable by histological staining.

Ovarian follicles are classified according to their size and diameter as large yolky follicles (Etches, 1993). These small follicles supply the growing follicles for

maintenance of the hierarchy. With the exception of the large yellow follicles, the rest of the follicles are regarded as small follicles. Fewer small follicles were found in the ovaries of aging hens and follicular atresia was common among these small follicles (Waddington et al., 1985; Palmer and Bahr, 1992). Follicles enter into rapid growth phase when they are less than 9 mm in diameter and contain white yolk (Gilbert, 1971). The rate of follicular maturation was slower in aging birds than in young ones (Tanabe et al., 1981; Moudgal and Razdan, 1984; Johnson et al., 1986; Joyner et al., 1987; Palmer and Bahr, 1992). It is possible that changes that take place in the theca and granulosa tissues of the follicles transform the follicles from non-ovulable to ovulable stage in the hierarchy and that this could be responsible for follicular maturation. However, it was suggested that as laying hens age, longer time is taken in transporting yellow yolk into the small growing ovarian follicles (Gilbert, 1971; Williams and Sharp, 1978). Etches (1996) and Oguike (2004) reported that within 14 days of molt induction, the population of large yellow follicles became reabsorbed and the ovary regressed, containing many small follicles. The differences in the sizes of yolky follicles, the changes in the way in which yolk accumulate into the follicles, the high rate of atresia in the small follicles and the slow recruitment of follicles into rapid growth phase between the old and young hens could be among the factors that initiate the gradual decline in egg production in a flock of aging birds. Su et al. (1995) reported increased number of small follicles following induced- molt of albino and non-albino old layers as well as increase in the growth intensity of the follicles after induced-molt. (Kuenzel, 2003). In chickens, regression of the ovary and oviduct during molting is achieved through apoptosis (Heryanto et al., 1997b). On fasting, ovary becomes regressed with a decline in estradiol and progesterone (Etches et al., 1984). However, the exact mechanism of apoptosis in regressing reproductive tissue is still not known. Giampauli et al. (2005) showed that a decrease in reproductive system activity causes regeneration of the glands of the uterine mucosa. Therefore, post-molting results are associated with organ regression levels obtained during the molting process (Ruszler, 1998). Also, the reduction in the ovary weight depends on the duration of fasting or body weight loss (BWL) level (Berry, 2003).

At peak laying periods the ovary of the domestic hen contained 30-100 small yolky follicles with diameters varying between 1 and 8mm. In general, the number of these

healthy follicles decreased with increasing size in that there were about 20 follicles with a diameter of 1-2mm and 1 follicle (mean<1) with a diameter of 7-8mm. The number of follicles with diameters >8mm (the hierarchy of large, yolky follicles) varied between 4 and 7. No information was obtained for growth of smaller follicles between 1 and 3mm. High zinc diet caused a marked 80% reduction in ovary weight by Day 10. The oviduct, although less affected, was still reduced approximately 60% in weight after feeding either 10gm Zn or 20gm Zn/Kg feed for 4 days. Seasonal reproduction in birds is marked by activation of the hypothalamo-pituitary-gonadal axis (Leska and Dusza., 2007). Adenohypophysis activity under hypothalamic control regulates annual cyclic changes in gonads (Benoit, 1962). Changes in cell pituitary populations of Nothura maculosa relative to the annual cycle have been previously determined (Sonez et al., 1995, Sonez and Von Lawzewitsch, 1997) but they were not correlated with ovarian histology. Park et al. (2004a) noted no differences in ovarian weight between hens undergoing feed withdrawal and hens fed 1% zinc either as Zn acetate or Zn propionate. The intermediate ovarian weight reduction in zinc fed hens vs feed withdrawal might be the result of differences in environmental conditions.

ORGAN WEIGHT AND DEPOSITION

The liver is the target organ of yolk phospholipoprotein synthesis, which is dependent on ovarian steroids, primarily estrogen (Sturkie, 1976). For birds receiving zinc-containing diets, liver weight loss might not occur, as glycogen in the liver no longer becomes depleted because of zinc interference with insulin secretion (McCormick and Cunningham, 1984b). Relative to the kidney, the accumulation of zinc in liver was considerably higher in all molted hens and unmolted hens. There were approx 3.1-fold and 3.2-fold increases in the hepatic zinc concentrations from Zn acetate-fed hens and Zn propionate- fed hens compared to that of feed-withdrawal hens. Park et al 2004b reported zinc concentrations in the kidney and liver of hens fed 1% zinc as Zn acetate or Zn propionate resulted in 3.7-fold and 5.3-fold and 11-fold and 12-fold, respectively. The accumulation of zinc in various tissues is dependent on tissue type and zinc feeding (McCormick and Cunningham, 1987). Cormick and Cunningham (1984b) observed a 4-fold, 10-fold, and 27-fold increase in the concentration of zinc in the kidney, liver, and pancreas, respectively, after feeding either 1% zinc or 2%

zinc as Zn oxide for 4 days. A linear increase in Zn deposition was observed in bone, liver, and kidney with Zn supplementation. at the 80-ppm level compared with lower levels. (Sunder et al., 2008). Zinc acetate depressed the fresh weight/kg body weight of the kidneys, ovary and oviduct to a greater extent than the corresponding oxide treatments. (Gibson et al., 1986). Seven- six- and threefold increases in zinc concentration were found in pancreas, liver and kidney, respectively. Zinc accumulation was also high and almost identical in ovarian follicles F1 to F4 but slightly less in F5 and F6 follicles. (Verheyen et al., 1990). There was extensive accumulation of zinc in kidney, liver, and especially pancreas after 4 days of feeding either 10gm Zn or 20gm Zn/Kg feed. The level of dietary zinc had no effect on the extent of accumulation in any tissue (Golden et al., 2008).

RATIONALIZATION FOR INDUCED MOLTING IN COMMERCIAL LAYERS BIRDS.

Induced molting can be justified on the basis of its positive effects in the form of enhanced productivity, reduced costs and industry investments in breeder farms, rearing farms, and hatcheries. Economic and welfare benefits of an induced molt include the extended use of approximately 50% fewer hens in commercial egg flocks with fewer male chicks hatched (Bell et al., 2004). Induced molting improves the post-molt performance of the laying hens when compared to the pre-molt performance. The improvements include the larger eggs, improved shell quality and enhanced rate of egg production. The internal egg quality also markedly increased as measured by the Haugh units (Zimmermann et al., 1987; Yousaf, 2006a; Yousaf, 2006b). Induced molting also increased egg production, the number of intact eggs and egg mass and decreases egg breakage, mortality and culling (Bar et al., 2001). The most significant difference in different egg production cycles is for the egg weight and number. Egg weight remains below 60gm up to 20 weeks in the first production cycle. During earlier weeks of laying, egg weights average 52.3gm as compared to 62.6gm and 63.4gm per egg in first, second and third production cycles, respectively. Egg weights are 57.4, 63.4, and 63.7gm per egg for the three cycles at the 35^{th} week of production (Bell, 2003). In laying hens age-related declines in shell quality and egg production, cessation of egg laying can be minimized by induced molting which

involves feed and light restriction. Induced molting prolongs productive life of flocks and provides effectives means of use of resources with enhanced returns on investments. In good economic conditions the useful life of a laying flock may be extended from less than 80 weeks to 110 weeks or even to 140 weeks through the careful use of the molting process. Induced molting is used to optimize pullet replacement on commercial layer farms. With current management systems, farms using the single-cycle (non molted) program would require 8.4 new flocks per layer house over a 10-year period, whereas only 5.7 flocks would be required for a typical two-cycle (one molt) flock system (Bell, 2003). Egg quality would suffer when egg producers push their flock's ages beyond the limits of good egg quality because egg producers have less flexibility to adjust production to meet the market demand (Bell, 2003; Yousaf and Chaudhry, 2008).

Molting hens should always have access to a nutritious and palatable maintenance diet, until the hens reach a pre-production physiological state. The academics, industry and the scientific community research have demonstrated that a return to the pre-production physiological state can be achieved by providing a maintenance diet. This diet is designed to bring the flock into a non-laying period and rejuvenation of oviduct (UEP, 2005 and Yousaf and Chaudhry, 2008). The commercial egg industry depends on molting to minimize rearing and replacement of pullets and depopulation of spent hens (Bell, 2001). Induced molting in flock management practice has increased the profitability of egg production (Bell, 2001 and Golden et al., 2008). More research is needed to understand the intestinal absorption mechanism involved in zinc uptake and how this might be used to enhance molt induction for much lower dietary levels of zinc than those that have been studied previously (Park et al., 2004d). Venkata et al. (2008) reported a number of studies conducted in an attempt to develop effective methods to molt hens without the use of feed removal, like high zinc in diets (Berry and Brake, 1987; McCormick and Cunningham, 1987) and low sodium diets (Whitehead and Shannon, 1974).

Complete removal of feed for a period of time is a traditional approach for inducing molt, but this method has come under increasing scrutiny due to physiological and food safety issues. Alternatives to feed withdrawal method have been examined historically but now it has become necessary need to replace feed withdrawal

procedure as an immediate issue. The development of successful alternative diets has been evaluated on basis of their effectiveness to limit S. enteritidis colonization but as animal welfare becomes more of a concern these diets will need to be re-examined for their ability to influence animal behavior in a positive fashion when compared to feed withdrawal responses. In addition, physiological, bone metabolism and neurological responses may prove to be important parameters to measure and evaluate respective alternative molting approaches. Several alternative molting diets have studied to be effective in preventing or at least limiting initial S. enteritidis colonization in the laying hen gastrointestinal tract (Park et al., 2004e).

Induced molting is a world-wide practice in poultry industry which significantly increases the productive lives of laying hens and profits of poultry farmers. However, due to the hen welfare concerns regarding the commonly used feed withdrawal method, this technique of molting has become controversial. Poultry farmers will always need to molt their flocks and for this reason they would welcome a more desirable method of molting to maintain a well managed, cost-effective and sustainable egg production system. Therefore, it is essential to test and validate the suitability of a non feed removal method by examining the most appropriate levels of feed restriction, photoperiod and feed additives for inducing molt and improving subsequent hen welfare, egg production and economics (Park et al., 2004c). Although several studies have been conducted to investigate effects of individual molting programs on either production parameters or immune responses, direct comparisons of effects of the available molting programs, simultaneously, on production parameters and reproductive profile have not been done. Present study was an attempt to evaluate different methods like high level zinc oxide in the diet and total feed withdrawal to induce molting in young and old White Leghorn layers birds.

The current study was conducted to validate the various methods like high doses of zinc oxide in the feed and total feed restraint to induce the molting and to determine the toxicity in white Leghorn layer birds and their effects on body weight, reproductive organs, egg production, hormonal profile, histological changes in gonadal axis and accumulation in different organ tissues. The zinc-induced feed intake suppression and the changes in corticosterone levels were analyzed. This study also provides histological criteria for the identification of atretic follicles, follicular

morphometric information and a simpler integrated view regarding the types of atresia. The other objective of the study was to investigate the changes in the number of small ovarian follicles and subsequent egg production of old laying hens as influenced by induced molting. The applicability of zinc supplementation and complete restrained feeding as a force-molting method based on anorexia and to study whether the changes induced through these methods are an irreversible inhibitory effect on the reproductive functions after regression that results from molting.

MATERIALS AND METHODS

The present study was conducted on eighty (80) single-comb White Leghorn (WLH) birds of two different ages (23 weeks and 67weeks). These birds were divided into two Batches according to their age, each comprising 40 chicks. Two experiments were designed, one for chicks of younger age and the other for older age chicks. Each experiment was based on four groups, i.e, control, off fed, zinc treatment with a dose of 25,000ppm and 30,000ppm. These experiments were carried out to investigate the effects on body weight, ovary (weight, length and width), ovarian histology, oviduct (length and weight), liver weight and blood serum hormonal profile for estradiol, progesterone, cortisone and cholesterol due to different treatments.

Experiment-I. The chicks in this experiment were of 23 weeks age.

Experiment-II. The birds in this experiment were of 67 weeks age

EXPERIMENT-I CHICKS OF 23-WEEKS AGE

This experiment was carried out using forty (40) healthy birds at pre-laying stage (21 weeks of age). The birds were purchased from a poultry farm and reared at Primate Facility, Department of Animal Science, Quaid-i-Azam University, Islamabad. The birds were allowed for two weeks to acclimatize themselves to their surroundings. The birds were housed in pens of 6x4 feet size which were cleaned and fumigated before use. The chicks were facilitated with dry rice hulls floor bed. Each pen was covered and separated by 3 feet high partition to avoid the mixing of birds. All the birds used in experiment were of the same age and weight. The birds were maintained under uniform standard management conditions in accordance with Ross Parent Stock management manual, 1995.

At the age of 23 weeks the chicks were randomly divided into four equal groups (N=10) in each group. The moulting like conditions was created in experimental chicks by two ways, by complete removal of feed and administration of high concentration of zinc in feed. The experimental plan was as follows.

Group I: Control

Group-I was fed normal at rate of 120gm/birds/day and water (Nonmolted).

Group II: (Without feed)

This group was kept off fed (feed restricted) but water was provided ad-libitum (Negative Control) for twelve days to induce molting (feed withdrawal method, Bell, 2002) with little modification in duration of treatment.

Group III: (25,000ppm Zinc/kg feed)

Molting was induced by administration of high dose of zinc as described earlier by Berry and Brake (1985). The hens in Group-III were given feed supplemented with Zinc oxide analytical grade (Merck) at the concentration of 25,000ppm Zinc/kg feed and birds were given prepared feed at the rate of 120gm/bird/day. This concentration of zinc was prepared by the addition of 34.11gm of Zinc oxide to 965.89gm of normal feed to get one kg Zinc mixed feed. The total duration of zinc treatment was twelve days.

Group IV: (30,000ppm Zinc/kg feed)

The chicks were given zinc 30,000ppm Zinc/kg for twelve days. Birds were fed prepared feed at the rate of 120gm/bird/day. In Group-IV for the preparation of one kg zinc mixed feed an amount of 37.34gm Zinc oxide was added to 962.66gm of normal feed.

Batch-I (Chicks slaughtered at 12^{th} day)

All the treated groups, including control, were examined for 12-days to record information as mentioned above. After 12-day treatment five chicks from each group were slaughtered after recording information. From these slaughtered chicks information regarding ovarian weight, length and width; oviductal weight and length and liver weight was recorded. For histological study ovarian tissue was processed further for section cutting.

Batch-II (Chicks slaughtered at 27^{th} day)

The remaining five birds were maintained for another fifteen days. In this part of the experiment some changes were made. Off fed condition and treatment with two Zinc oxide dosages were withdrawn. In all the four groups the chicks were fed on normal feed at the rate of 120gm/bird/day for a period of 15-days. After this period these five chicks were also slaughtered and information regarding ovary, oviduct and liver were recorded as was done with Batch-I.

Composition of Normal Feed

The feed provided to chicks contained 89.48% dry matter, 10.53% moisture, 14.66% crude protein, 4.38% fat and 9.13% total mineral (Ash) and 5-15 PPB Alfa toxin as was analyzed by feed testing laboratory, Poultry Research Institute (PRI) Rawalpindi. Birds were frequently observed to detect any clinical problem. No clinical problem was detected or checked throughout the experiment. In Batch-II of both experiments same feed was given to birds without addition of zinc oxide.

TEMPERATURE AND LIGHTING SCHEDULE

Experimental birds including control group were kept at controlled temperature (22-24^{o}C) and a relative humidity (54-78%) following Kaya et al (2001b) and Barlett et al (1990). Egg production by layer birds depend upon photoperiod. During first part of the experiment for the period of 12-days photoperiod was maintained at 14 hour light: 10 hour dark (14hL: 10hD) period. In this second part of the experiment (extended further for 15-days) the light and dark schedule was gradually changed. The light period was increased gradually up to 16h light : 8h dark (16hL : 8hD) to stimulate the egg production (Campo1 and Da´vila, 2002). This photoperiod was maintained up to the end of the experiment. The intensity of light is also very important to stimulate the pituitary gland for initiation of egg formation process through gonadotrophins production, so it was adjusted at 1 watt/4cm3 or 5 lux (Ernst, 1998; Anjum, 1997).

EXPERIMENT-II CHICKS OF 67 WEEKS AGE

The design of this experiment was the same as with experiment-I. In this study forty (40) healthy single-comb White Leghorn (WLH) chicks were used and their average age was 67 weeks after their first production. Experimental birds were purchased from a commercial layer poultry farm located at Thanda Pani in Islamabad. A period of two weeks was given to the birds to acclimatize to their surrounding before the start of experiment.

MOLTING PROCEDURE AND LIGHTING PLAN

At the age of 67 weeks the birds were randomly divided into four groups comprising ten (10) birds in each group. In these four groups same procedure of treatment was followed as in the first experiment

Group I: Control

Fed normal diet at the rate of 120gm/birds/day and water (Non-molted)

Group II: (Without feed)

Kept on restraint feeding (Negative Control) and provided with water ad-libitum. Molting was induced in this group by the feed withdrawal method (Bell, 2002) with little modification in duration of treatment.

Group III: (25,000ppm Zinc/kg feed)

Zinc enriched feed was prepared in the same way as in the first experiment and birds were given prepared feed at the rate of 120gm/bird/day. Molting was induced by administration of high dose of zinc as described earlier by Berry and Brake (1985) with some alteration in concentration of zinc used.

Group IV: (30,000ppm Zinc/kg feed)

All chicks received 30,000ppm Zinc per kg feed for twelve days at the rate of 120gm/bird/day.

Batch-I (Chicks slaughtered at 12^{th} day)

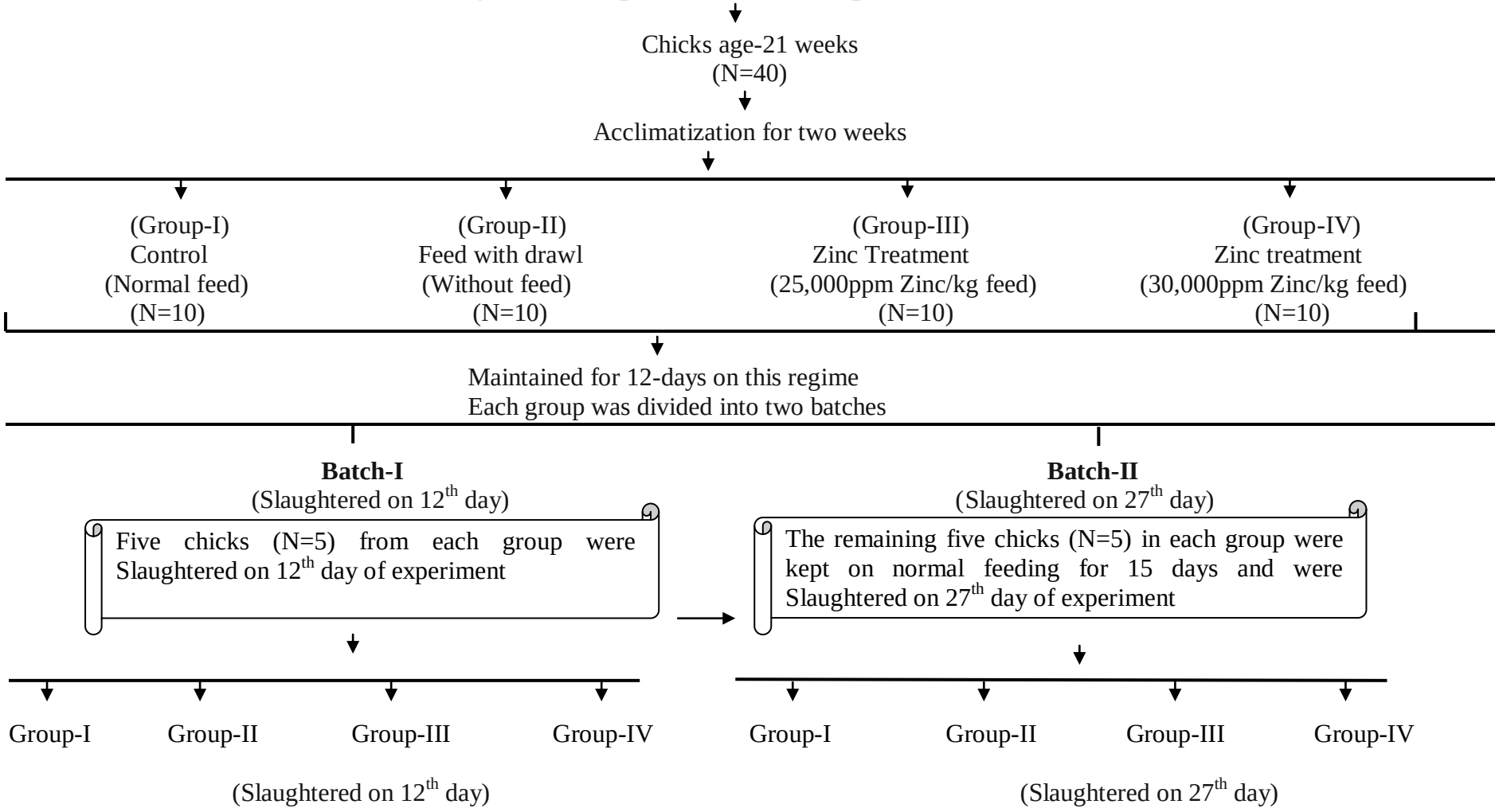
All the treatment groups, including control, were examined for 12-days to record information as mentioned above. Five chicks from each group were slaughtered on 12^{th} day of treatment and same information was recorded as the in first experiment.

Batch-II (Chicks slaughtered at 27^{th} day)

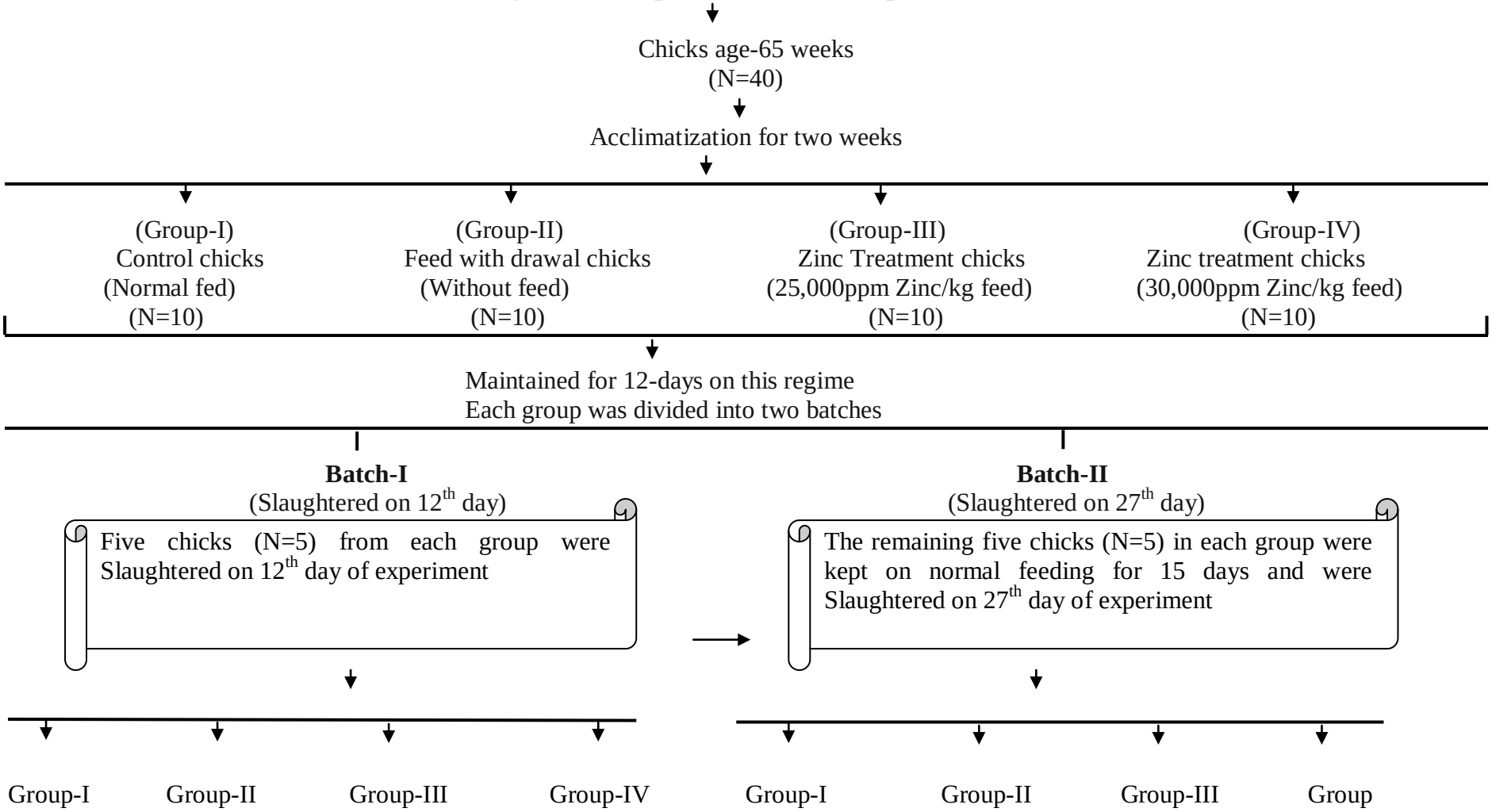
The remaining chicks were slaughtered after extended 15 days for recording information as mentioned earlier. Off fed condition and treatment with two Zinc oxide dosages were withdrawn. In all the four groups the chicks were kept on normal feed at the rate of 120gm/bird/day for a period of 15-days as in Batch-II of experiment-I.

Lighting schedule in the first half of the experiment was (14hL : 10hD) and (16hL : 8hD) light and dark period was maintained in the second half of the experiment (Sundaresan et al., 2008a; Park et al., 2004c). The reason for this light and dark period has been mentioned in first experiment.

Diagrammatic presentation of Experiment No. 1



Diagrammatic presentation of Experiment No. 2



GENERAL METHODS FOR BOTH EXPERIMENTS

Following are the general methods for both experiments.

FEED-INTAKE RECORDING AND BODY WEIGHT

During zinc-induced molting feed intake was recorded in individual birds from the first day of experiment up to the end of the experiment to determine the level of feed-intake reduction. Five birds from each group were sacrificed on 12^{th} day of molting (DOM) and five birds were slaughtered on 27^{th} day of experiment. The body weight of each bird was measured at an interval of two days from first day of experiment till the end of whole experiment to determine the increase in body weight with the age of the birds.

COLLECTION OF BLOOD SAMPLES

Blood samples were collected from each bird between 5:00 PM-7:00 PM to minimize the handling stress which if not minimized can affect serum hormonal levels (Maho et al., 1992; Dehnhard et al., 2003). Blood samples were taken from superficial vein puncture of the wing (brachial) vein starting from start of experiment on every alternate day intervals in sterilized 3ml heparnized syringes fitted with 24 gauge needle and continued until the end of experimental period in both experiment. Blood samples were then transferred in to pre-sterilized test tubes and plasma samples were separated by centrifugation (3000Xg for 10-15 minutes). Plasma samples were stored at $-20^{o}C$ until used for the estimation of estradiol, progesterone and corticosterone by Enzyme-linked immunosorbent assay (ELISA).

EGG PRODUCTION

During the experimental period, the egg production was recorded daily in each group. Egg production was calculated in terms of group day (percent) and calculated by egg laid by that group divided by number of hen in that group.

TISSUE SAMPLING

The ovaries, oviducts and liver were dissected out and the organ's size was measured by vernier calipers. All the organs were weighed to the nearest gm with Sarotoreious Digital Balance. Ovary containing ovarian stroma and various types of follicles,

oviduct and liver from control birds and treated animals were collected for routine histology and some part of these tissues from all the groups were stored separately at -70 ^{0}C for the estimation of zinc absorption by atomic absorption spectrometry.

HISTOLOGICAL AND MORPHOMETRIC STUDY

After slaughtering the birds by Muslim method (Gracey and Collins, 1992), tissues of ovary, oviduct and liver were removed quickly and fixed in sera fixative (Alcohol=60ml, Formalin=30ml and Glacial acetic acid=10ml) for 4-5 hours. Then tissues were dehydrated in ascending grades of alcohol in subsequent steps. After dehydration tissues were transferred to cedar wood oil until they became clear and transparent. These tissues were embedded in paraplast by the following procedure.

Benzol I = 10 minutes (At room temperature)

Benzol II = 10 minutes (At room temperature)

Benzol + Paraplast = 20 minutes at 60 ^{0}C

Paraplast I = 12 hours at 60 ^{0}C

Paraplast II = 12 hours at 60 ^{0}C

Paraplast III = 12 hours at 60 ^{0}C

The tissues embedded in paraffin were fixed on wooden blocks for section cutting with a rotatory microtome (Shandon, Finesse 325) at a thickness of 5µm and stretched at 60^{o}C on egg albumin coated slide on fisher slide warmer for 24 hours so that ribbon stretched and firmly attached. These slides were transferred to xylene 1 and xylene 2 for 15 minutes each to remove the wax. The tissues were hydrated in the descending grades of alcohol, washed in tap water and stained with Harris Hematoxylin. The slides were placed in tap water until nuclei became blue. After that tissues were rinsed in tap water and dehydrated with ascending grades of alcohol, counter stained with Eosin (Mcmanus and Mowry, 1960). The stained section showing different stages of oogenesis were studied under light microscope (Nikon, 238483) with ocular micrometer at different magnification for morphometric analyses. Microphotography was done by microscope (Leica DM LB2/11888111) equipped with Cannon Digital Camera (S50 8434401812).

HISTOLOGICAL EVALUATION OF FOLLICULAR HEALTH OR ATRESIA

Five birds from each group were sacrificed on 12th day of experiment and remaining five birds in each group in both experiments were slaughtered on 27th day of experiment to collect the ovary and oviduct. All yolky follicles were measured, counted and yolk present in all the follicles was drained out.

The prepared slides from middle portion of ovarian samples from all the birds of each group were studied histologically. The ovarian follicles were examined for atretic changes as described earlier (Yoshimura et al., 1989; Yoshimura and Tamura, 1985). Briefly, the specimens were evaluated for follicular health or atresia. The healthy follicles were those with intact membrana granulosa and a few pyknotic nuclei in this layer. Alternatively, atretic follicles were evident with their attenuated membrana granulosa, loosely attached granulosa cells and increased number of pyknotic nuclei. For morphometric analysis, diameter of primary follicles, developing follicles, non yolky large and small follicles and thickness of follicular epithelium including granulosa and theca was also measured. Cellular structure and arrangement was observed in thecal layer and granulose layer. Five ovarian tissue slides of each bird in control and treated group were selected and ten cross sections from every slide were morphometrically analyzed. So fifty (50) cross sections per animal were evaluated for statistical observation. The mean follicles number, their diameter and thickness was calculated per bird per ovarian section for both control and molted birds.

ESTIMATION OF ELEMENTARY ZINC RESIDUE

In current study, deposition of elemental zinc content was estimated in ovary, kidney and liver of control and all treated birds by atomic absorption spectrophotometer method. A piece of each of these organs from each animal in all groups was used for the estimation of zinc by atomic absorption. 0.5 g (frozen) sample of ovary, kidney and liver was dried at 80 ^{0}C for 3 days in hot oven and subsequently predigested in 5ml concentrated nitric acid for 3 days. The predigested sample was further digested for 2h at 100 ^{0}C (Davis, 1998; Daugherty, 2002) using a VWR oven (Model#1305U-2). The temperature was lowered down to the room temperature after 10 minutes. The digested samples were filtered and diluted by adding 10ml of deionized water, making the final volume of 15ml. The same deionized water was used for blanks. Then zinc

concentration was estimated using hydride generating fitted with flame Fast Sequential Atomic Absorption spectrophotometer (Varian AA240FS) at wavelength 193.7um, slit (nm) 0.5, relative sensitivity 1, relative intensity 50 and current was 9mA. The results were expressed as µg/mg of tissue of the ovary, kidney and liver samples.

HORMONE ESTIMATION

Hormonal analysis was carried out for the estimation of plasma levels of steroid hormone in layer birds at 25 and 67 weeks of age. ELISA kits (Aumgnix Inc) were used for the determination of estradiol, progesterone and corticosterone concentration in blood plasma.

ELISA FOR THE MEASUREMENT OF ESTRADIOL IN BLOOD SERUM

Assay Procedure

All the coated wells 25µl of standards specimen and controls were dispensed. Estradiol-HRP Conjugate Reagent (100µl) and 50µl of rabbit anti-Estradiol (E2) reagent was added to each well and it was incubated for 90 minutes at room temperature (18-25^{o}C). After incubation the microwells were washed five times with deionized or distilled water in an automated washer (Platos W96, AMP Diagnostic). In each well 100µl of TMB Reagent was dispensed and incubated for 20 minutes at 18-25^{o}C. The reaction was stopped by adding Stop Solution to each well. The absorbency was read at 450nm and the results were expressed in pg/ml.

ELISA FOR THE MEASUREMENT OF PROGESTERONE IN BLOOD SERUM

Assay Procedure

All the coated wells 25µl of standards, specimen and controls were dispensed. Working Progesterone-HRP Conjugate Reagent (100µl) and 50µl of rabbit anti-Progesterone (P2) reagent was added to each well and it was incubated for 90 minutes at room temperature (18-25^{o}C). After incubation the micro wells were washed five times with deionized or distilled water in an automated washer (Platos W96, AMP Diagnostic). In each well 100µl of TMB Reagent was dispensed and incubated for 20

minutes at 18-25^{o}C. The reaction was stopped by adding Stop Solution to each well. The absorbency was read at 450nm and the results were expressed in pg/ml.

ESTIMATION OF CORTICOSTERONE

Serum corticosterone was estimated from control and treatment birds at various intervals by using an enzyme immunoassay kit (Diagnostic System Laboratories, Tex., USA) according to the manufacturer's instructions.

STATISTICAL ANALYSIS

The data in tables and text are presented as Mean ± SE (Number of samples) for control and different treatment groups. Differences were compared by analysis of variance using computer program Graph PadPrim version 5.00 (www.Graphpad.com) and comparisons between individuals were made by the student t-test. Linear Regression analysis of variance was used to find the duration dependent trend by evaluating the means from data of all the groups against both methods of treatment used in present experiment. A probability (P) value less than 0.05 was considered as significant difference.

The present experiments were designed to investigate the various methods like high doses of zinc oxide in the feed and complete feed restraint to induce the molting and to determine their toxicity in white Leghorn (WLH) layer birds at two different ages. For this purpose two experiments were conducted on two different age groups of the birds.

Experiment-I. The chicks in this experiment were of 23 weeks age.

Experiment-II. The birds in this experiment were of 67 weeks age

EXPERIMENT-I

Experiment I was started with ten birds at the age of 23 weeks in each of the four groups which were maintained during the experiment as follows.

Group-I was fed normal feed and designated as control group.

Group-II birds were not fed for 12-days and kept on drinking water only

Group-III birds were treated with a dosage of 25,000ppm zinc/kg of feed for 12-days

Group-IV birds were treated with a dosage of 30,000ppm zinc/kg of feed for 12-days

After 12-days of treatment, five birds of each group were slaughtered and observations were recorded as detailed in each group. The slaughtered birds were designated as BATCH-I (12- DAYS BATCH).

The remaining five birds were fed on normal feed and slaughtered after 27-days of the start of experiment. They were neither kept off fed (Group-II) nor were they treated with zinc doses. The required observations were recorded, which are detailed in the respective groups. This part of the experiment was designated as BATCH-II (27-DAYS BATCH).

BODY WEIGHT

Body weight in each group was measured at an interval of two days and is presented in Table 1. At 12^{th} day of experiment mean body weight was also compared with control (Group-I) and within all groups (Fig. 1). Initial mean body weight in each group was compared with the final weight at 12^{th} day of experiment (Fig. 2).

Group-I (Control)

Control group was fed on normal diet for twelve days and after two days interval their individual body weight was recorded. At the start in this group mean body weight of the ten birds was 1139.00±51.91gm and at day 12 their mean weight was 1245.00±45.02gm.There was non significant increase ($t_{(18)}$=1.54; P<0.14) in mean body weight at 12th day in this group compared to initial body weight. The linear regression analysis of variance indicates that weight gain was highly significant over the period of 12-days (b=29.40±3.78; $F_{(1,3)}$=60.50; P=0.004).

Group-II (Off Fed)

The animals in this group were kept only on drinking water without feed for twelve days. Mean body weight loss (gm) in each animal over the 12-days in ten birds was recorded at an interval of two days. Due to off fed condition there was constant weight loss in these animals. At the start of this experiment mean body weight of these animals was 1164.67±43.72gm and at day twelve this was 1001.11±36.67gm. Loss of mean body weight after 12-days in this group was highly significant ($t_{(18)}$=2.90; P=0.001) compared to initial mean body weight.

The regression analysis of variance on 12th day showed significant loss of mean body weight (in other words growth retardation) (b=-40.44±1.491; $F_{(1,3)}$=735.8; P=0.0001).

Group-III (25,000ppm Zinc/Kg Feed)

In this **group ten** animals were administered with a zinc dose of 25,000ppm/kg feed. The experiment was proceded further in the same way as in Group-I and Group-II. Mean body weight of each animal was recorded at an interval of two days. There was a meager decrease mean body weight on 12th day of experiment compared to in initial mean body weight but this difference was not significant ($t_{(18)}$=1.32; P=0.20) The regression analysis of variance shows that effect of zinc resulted in highly significant decrease in body weight by day twelve (b=-21.35±1.94; $F_{(1,3)}$=121.80; P=0.002).

Group-IV (30,000ppm Zinc/Kg Feed)

In this group ten birds were kept on diet mixed with zinc at a dose of 30,000ppm/kg feed for 12 days. Individual weight of all birds was recorded on an interval of two days. There was non significant decrease ($t_{(18)}$=1.99; P=0.07) in mean body weight on

12^{th} day of treatment (1170.00±21.78gm) compared to initial mean weight of the birds (1211.00±54.01gm). The regression analysis of variance shows that zinc resulted in highly significant decrease in body weight over the period of twelve days (b=-35.85 ± 4.61; $F_{(1,3)}$= 60.43; P=0.0044).

Comparisons

Mean body weight gain/loss on 12^{th} day were compared among the four Groups. Group-II ($t_{(18)}$=4.25; P=0.001) and Group-IV ($t_{(18)}$=2.94; P=0.01) showed significant decrease while Group-III (25,000ppm Zinc) resulted in non significant decrease ($t_{(18)}$=2.04; P=0.057) in mean body weight on 12^{th} day compared to Group-I (control). There was significant decrease in mean body weight in Group-II compared to Group-III ($t_{(18)}$=2.23; P=0.039) but there was no significant difference ($t_{(18)}$=0.47; P=0.64) when compared to Group-IV. Non significant difference ($t_{(18)}$=1.28; P=0.22) was noticed in mean body weight between Group-III and Group-IV.

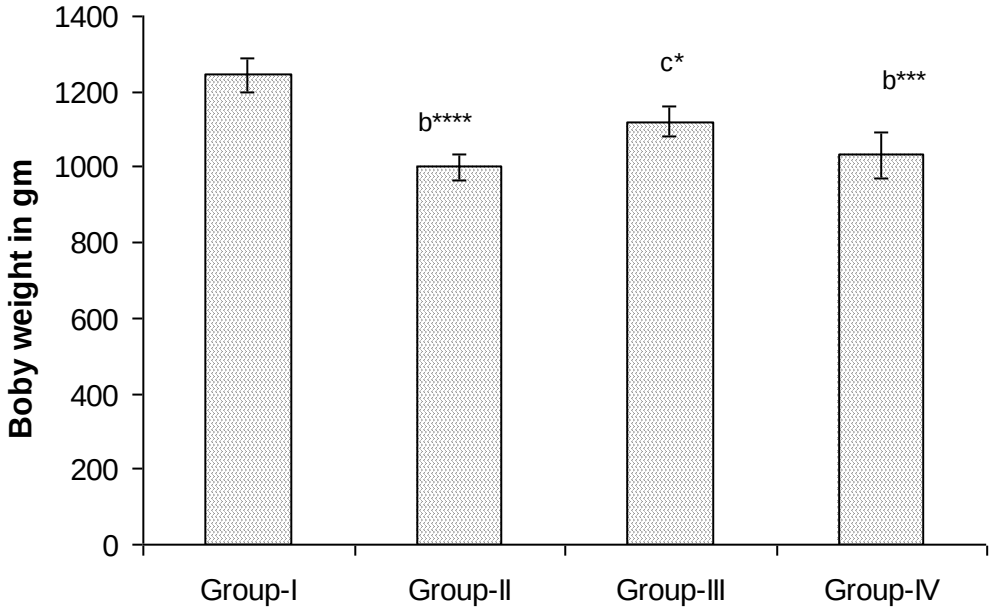


Fig 1: Effect of restraint feeding and zinc administration on mean body weight (gm) in control and treated groups of young White Leghorn (WLH) layer birds on 12th day of experiment (N=10).
b=Group-II compared to Group-III and group-IV at 12th day
c=Group-III compared to Group-IV at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

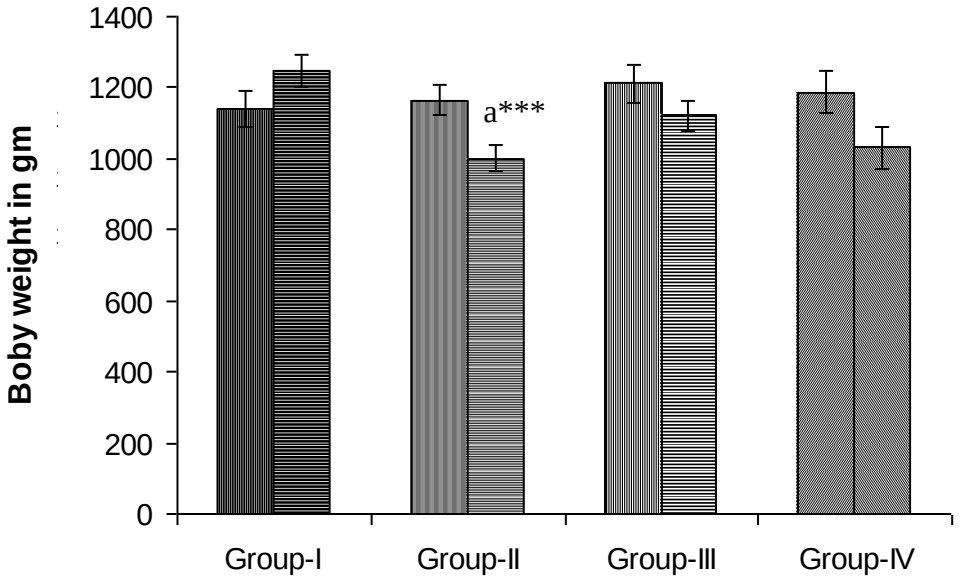


Fig 2: Comparison of initial body weight and body weight (gm) on 12th day of restraint feeding and zinc administration in control and treated groups of young White Leghorn (WLH) layer birds (N=10).
a=Group-I compared to all other groups at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

BATCH-I

1. BODY WEIGHT

Body weight in each group was measured at an interval of two days and is presented in Table 2. At the 12th day of experiment mean body weight of all treatment groups was also compared with control (Group-I) and within all groups (Fig. 3). Initial mean body weight in each group was compared with the final weight at 12th day of experiment (Fig 4).

Group-I (Control Group)

Mean body weight of five birds slaughtered on 12th day of experiment was recorded on every two days interval given in Table 2. There was non significant gradual increase ($t_{(8)}$=1.46; P=0.18) in body weight on 12th day. The regression analysis of variance showed that weight gain was highly significant (b=27.00±2.810; $F_{(1,3)}$=92.36; P=0.0024) over the period of 12 days.

Group-II (Off Fed)

As in Group-I (control) five birds were slaughtered after 12-days (Batch I) maintained in Group-II are presented in Table 2. The mean body weight of these five birds was 1144.40±83.46gm initially and 972.00±63.83gm on 12th day. Group-II showed non significant loss in body weight ($t_{(8)}$=1.64; P=0.14) compared to initial mean body weight.

The regression analysis of variance showed that there was highly significant decrease in mean body weight (b=-41.88±1.49; $F_{(1,3)}$= 792.6; P<0.0001) with the advance in time of off fed condition.

Group-III (25,000ppm Zinc/Kg Feed)

After 12th day of experiment five of the ten animals were slaughtered. Their mean body weights were also recorded (Table 2). There was non significant decrease ($t_{(8)}$=1.36; P=0.21) in mean body weight compared to initial weight of the birds. The regression analysis of variance showed significant decrease in mean body weight during the period of 12-days (b=-16.50±1.42; $F_{(1,3)}$= 135.4; P=0.0014).

Group-IV (30,000ppm Zinc/Kg Feed)

A non-significant decrease ($t_{(8)}$=2.29; P=0.051) in mean body weight was observed between the initial mean body weight and birds slaughtered on 12th day of experiment (Table 2). The regression analysis of variance regression analysis of variance after 12th day showed highly significant loss (b=-34.90±7.21; $F_{(1,3)}$=23.41;P=0.02) of body weight.

Comparisons

After 12 days of treatment difference in mean body weight was measured between initial body weight and on 12th day. Mean body weights of Group-II (off-fed), Group-III (25,000ppm Zn) and Group-IV (30,000ppm Zn) decreased sharply ($t_{(8)}$=4.61; P=0.002; $t_{(8)}$=5.76; P=0.0004 and $t_{(8)}$=6.77; P=0.0001) respectively, compared to Group-I (control). There were non-significant differences in mean body weights of Group-III ($t_{(8)}$=0.67; P=0.52) and Group-IV ($t_{(8)}$= 0.45; P=0.66) compared to Group-II. Similar trend in mean body weight of Group-III Vs Group-IV ($t_{(8)}$= 1.10; P=0.08) was observed.

Table 2: Effect of restraint feeding and zinc administration on mean body weight (gm) in control and treated groups of young White Leghorn (WLH) layer birds, slaughtered on 12th day of experiment (Batch-I)

Treatment groups	Group-I	Group-II	Group-III	Group-IV
Initial Body weight	1243.00±54.26	1144.40±83.46	1088.00±45.43	1096.00±61.04
3rd day	1260.00±59.41	1094.00±74.67	1067.00±48.44	1008.00±53.61
6th day	1289.50±50.31	1055.00±69.35	1049.00±48.85	994.00±54.64
9th day	1308.00±38.39	1020.00±71.48	1042.00±24.37	971.00±32.57
12th day	1354.00±52.97	972.00±63.83^{b***}	1018.00±23.38^{b****}	940.00±30.50^{b****}

Values are means±SEM (N=5)
a=Initial weight of that group compared to final weight.
b=Group-I compared to all other groups at 12th day
c=Group-II compared to Group-III and Group-IV at 12th day
d=Group-III compared to Group-IV at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

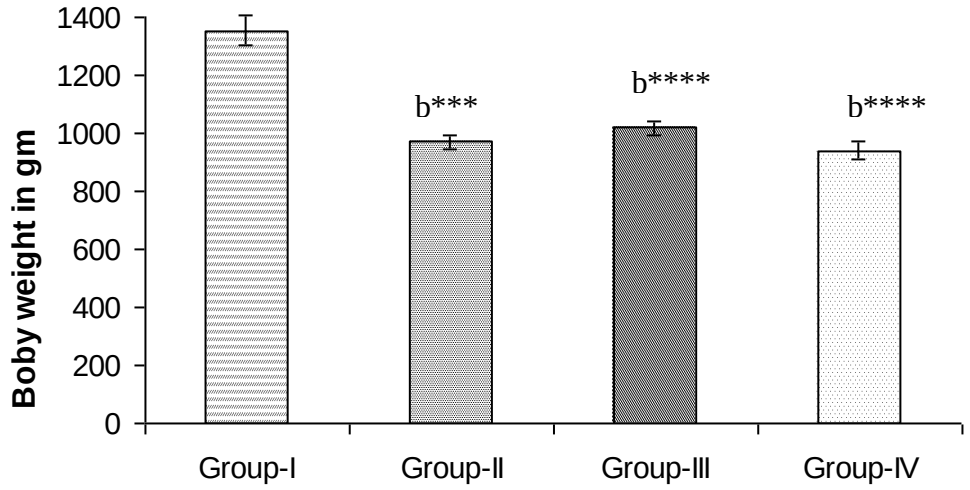


Fig. 3. Effect of restraint feeding and zinc administration on mean body weight (gm) in control and treated groups of young White Leghorn (WLH) layer birds slaughtered on 12th day of experiment (Batch-I)
b=Group-II compared to Group-III and group-IV at 12th day
c=Group-III compared to Group-IV at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

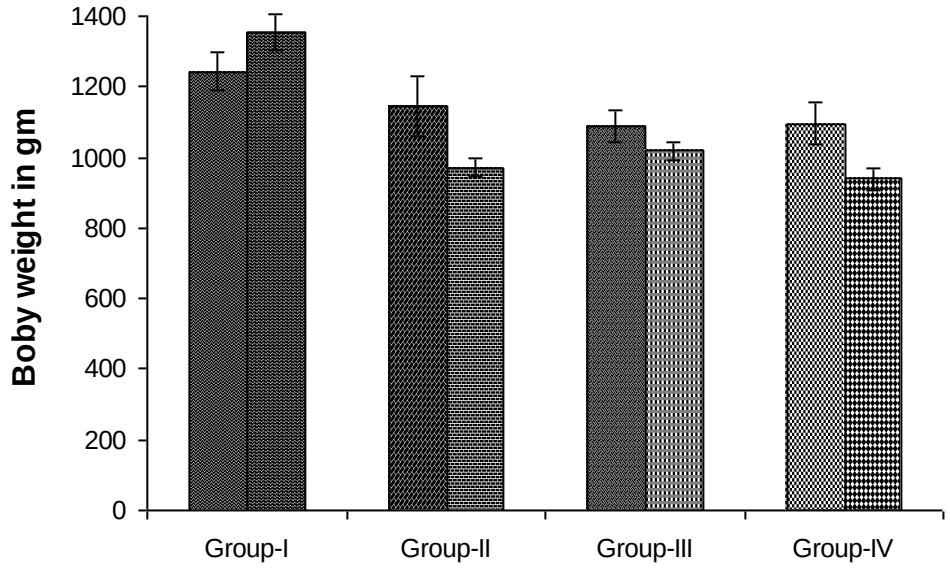


Fig. 4: Comparison of initial body weight and body weight (gm) in birds slaughtered on 12th day of restraint feeding and zinc administration in control and treated groups of young White Leghorn (WLH) layer birds.(Batch-I)
a=Group-I compared to all other groups at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

2. SECONDARY SEXUAL CHARACTERS

Combs and Wattles

Secondary sexual characters of the birds like comb and wattles were also examined in control and treated groups (Table 3).

Comb

After 12-days from start of experiment five birds (Batch-I) were slaughtered and their comb length and width and wattles length and width were recorded. The mean comb length ($t_{(8)}$=3.63; P=0.01) decreased significantly but width ($t_{(7)}$=1.23; P=0.25) reduced non-significantly when Group-II (off fed) was compared to Group-I. There was significant reduction in mean comb length ($t_{(8)}$=4.16; P=0.003) and width ($t_{(8)}$=2.72; P=0.03) in Group-III (25,000ppm Zn) compared to Group-I. In Group-IV (30,000ppm Zn/kg feed) mean comb length ($t_{(8)}$=3.70; P=0.01) decreased significantly and width ($t_{(8)}$=1.69; P=0.13) decreased non-significantly compared to Group I. There was no significant difference in mean comb length and width of Group-III (25,000ppm Zn/kg feed) compared to that of Group-II. Similarly, Group-IV compared to Group-II showed no-significant differences in mean comb length and mean comb width. No significant difference in mean comb length and width was seen in a comparison between Group-III vs Group-IV.

Wattles

In Group-II (off fed) wattles length ($t_{(8)}$=2.47; P=0.04) and width ($t_{(8)}$=3.98; P=0.004) significantly reduced compared to that of Group-I. While wattles length and width in Group-III (25,000ppm Zn/kg feed) and Group-IV (30,000ppm Zn/kg feed) did not reduce significantly compared to Group-I. No significant differences in mean wattles length and wattles width were seen in a comparison between Group-II with Group-III and Group-IV. Comparison of mean wattle length and wattle width between Group-III and Group-IV also showed no significant differences.

3 EFFECT ON OVARY, OVIDUCT AND LIVER

Mean ovarian weight, length and width, oviduct length and weight and liver weight of control and all treated groups were recorded Table 4.

The decrease in mean ovarian weight was highly significant in Group-II (off fed) ($t_{(8)}$=21.45; P<0.0001), Group-III (25,000ppm Zn/kg feed) ($t_{(8)}$=14.03; P<0.0001) and

Group-IV (30,000ppm Zn/kg feed) ($t_{(8)}$=16.49; P<0.0001) compared to Group-I (control). However, this decrease in ovarian weight was greater in off fed group (Group-II). Mean ovarian weight was significantly higher in Group-III ($t_{(8)}$=3.42; P=0.01) but no difference was found in Group-IV ($t_{(8)}$=2.26; P=0.053) when compared to Group-II. Group-III Vs Group-IV also showed no significant difference in mean ovarian weight.

Comparisons between Group-I Vs Group-II ($t_{(8)}$=2.61; P=0.03), Group-I Vs Group-III ($t_{(8)}$=2.36; P=0.05) and Group-I Vs Group-IV ($t_{(8)}$=2.52; P=0.04) showed significant decrease in mean ovarian length. Group-II compared to Group-III and Group-IV showed no significant change in mean ovarian length. There was also no difference in mean ovarian length in a comparison between Group-III Vs Group-IV. Mean ovarian width did not change significantly in all treated groups compared to control group.

The highest oviductal weight was in Group-I (43.69±3.14gm) and the lowest oviductal weight was in Group-IV (12.81±1.30gm). Compared to Group-I, there was significant reduction in mean oviductal weight in Group-II ($t_{(8)}$=7.87; P<0.0001), Group-III ($t_{(8)}$=7.75; P<0.0001) and in Group-IV ($t_{(8)}$=9.09; P<0.0001). There was non significant decrease in oviductal weight in Group-III and Group-IV compared to Group-II. No significant difference in oviductal weight in Group-III Vs Group-IV comparison was seen.

Mean oviductal length decreased significantly in Group-II ($t_{(8)}$=9.12; P<0.0001), Group-III ($t_{(8)}$=5.80; P=0.0004) and in Group-IV ($t_{(8)}$=12.48; P<0.0001) compared to Group-I. No significant difference was seen in oviductal length in comparison of Group-II Vs Group-III but in Group-IV ($t_{(8)}$=2.49; P=0.04) mean oviductal length was significantly decreased compared to Group-II. In Group-III Vs Group-IV a non significant decrease was observed in mean oviductal length of Group-IV.

The liver weight in birds kept off fed (Group-II) ($t_{(8)}$=6.61; P=0.0002) and Group-IV (30,000ppm Zn/kg feed) ($t_{(8)}$=2.93; P=0.02) showed significant decrease but liver weight in birds treated with 25,000ppm dosage of zinc (Group-III) did not decrease significantly compared to Group-I. Mean liver weight in Group-III ($t_{(8)}$=2.42; P=0.04) and Group-IV ($t_{(8)}$=5.35; P=0.0007) was significantly higher compared to Group-II. There was no significant difference in mean liver weight between Group-III and Group-IV.

Table 3: Effect of complete restraint feeding and zinc administration on mean comb and wattle length and width (mm) of young White Leghorn (WLH) layer birds, slaughtered after 12 days (Batch-I)

Treatment groups	Comb		Wattles	
	Length	**Width**	**Length**	**Width**
Group-I	70.84±1.83	37.4±1.50	27.10±0.84	22.73±1.17
Group-II	54.33±4.65[a***]	33.27±3.36	20.88±2.65[a*]	16.94±0.96[a***]
Group-III	54.97±3.35[a***]	30.43±2.08[a*]	25.14±1.92	19.71±1.76
Group-IV	54.82±3.93[a***]	31.52±3.14	22.88±1.67	18.41±1.54

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

4 ZINC DEPOSITION IN OVARY, LIVER AND KIDNEY

Zinc concentration (deposition) in kidney, liver and ovary was measured in all four groups of this experiment and is presented in Table 5.

Deposition Of Zinc In Ovary

In Batch-I there was significantly higher zinc deposition in the ovary of Group-III (25,000ppm zinc/Kg feed) ($t_{(8)}$=3.23; P=0.01) and Group-IV (30,000ppm zinc/kg feed) ($t_{(8)}$=4.69; P=0.002) but Group-II (off fed) showed no significant ($t_{(8)}$=2.04; P=0.08) change compared to that of Group-I (control). While sharp increase in zinc contents in mean zinc deposition was evident in Group-IV ($t_{(8)}$=3.95; P=0.004) and non significant increase in Group-III ($t_{(8)}$=1.83; P=0.10) compared to Group-II (off fed). The difference in zinc deposition in Group-III vs Group-IV was non significant ($t_{(8)}$=2.05; P=0.07).

Deposition Of Zinc In Liver

In the liver of the birds slaughtered after 12-days (Batch-I) zinc deposition in Group-I (control) and (off fed) Group-II ($t_{(8)}$=0.19; P=0.86) birds was similar however, those birds who were treated with 25,000ppm zinc dosage (Group-III) ($t_{(8)}$=24.64; P<0.0001) and 30,000ppm zinc dosage (Group-IV) ($t_{(8)}$=22.22; P<0.0001) have significantly higher mean zinc deposition than the Group-I (control). In Group-III (25,000ppm zinc) ($t_{(8)}$=24.58; P<0.0001) and Group-IV (30,000ppm zinc) ($t_{(8)}$=24.19; P<0.0001) administered high dose zinc showed highly significant deposition of zinc compared to Group-II (off fed). The zinc deposition in Group-IV which had received high dose of zinc was also significantly greater than in group III (lower dose) ($t_{(8)}$=7.12; P=0.0001).

Deposition Of Zinc In Kidneys

In Batch-I there was no appreciable difference in zinc contents in kidneys of Group-I and Group-II birds ($t_{(8)}$=0.08; P=0.94). Birds treated with zinc dosage 25,000ppm (Group-III) ($t_{(8)}$=7.67; P<0.0001) and 30,000ppm zinc dosage (Group-IV) ($t_{(8)}$=10.88; P<0.0001) showed highly significant zinc deposition compared to Group-I (control). Those birds which were treated with 25,000ppm zinc (Group-III) ($t_{(8)}$=7.60; P<0.0001) and 30,000ppm zinc dosages (Group-IV) ($t_{(8)}$=10.79; P<0.0001) also

showed highly significant zinc deposition compared to Group-II (off fed). Group-III vs Group-IV comparison showed a significant higher concentration of zinc deposition in the group which received high dose of zinc (Group-IV) ($t_{(8)}$=2.87; P=0.02).

Table 5: Mean Zinc Concentration (µg/g) in ovary, liver and kidney in young White Leghorn(WLH) layer birds slaughtered after 12 days of experiment (Batch-I)

Treatment groups	Batch-I (slaughtered after 12th day of experiment)		
	Ovary	**Liver**	**Kidney**
Group-I	1.24±0.55	11.86±0.64	2.27±0.22
Group-II	2.58±0.34	12.04±1.50	2.24±0.34
Group-III	$3.42\pm0.33^{a***}$	$95.72\pm3.16^{ab****}$	$15.01\pm1.65^{ab****}$
Group-IV	$4.44\pm0.40^{ab***}$	$144.04\pm5.60^{abc****}$	$22.01\pm1.80^{ab****c**}$

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

5 HORMONAL ESTIMATION

Hormonal analysis for mean plasma Estradiol, Progesterone, Corticosterone and Cholesterol is shown in Tables 6, 7 and 8 respectively.

Plasma Estradiol Level

The mean plasma concentration of estradiol in the different treatments is shown in Table 6. On day 3rd of treatment, the hens in Group-II ($t_{(18)}$=5.54; P<0.0001), Group-III ($t_{(18)}$=2.843; P=0.01) and Group-IV ($t_{(18)}$=3.33; P=0.004) showed lower levels of estradiol in their plasma compared to the level in Group-I (control). On day six (6) of treatment plasma level of estradiol of Group-II ($t_{(18)}$=5.65; P<0.0001), Group-III ($t_{(18)}$=5.24; P<0.0001) and Group-IV ($t_{(18)}$=6.02; P<0.0001) was also significantly decreased than Group-I (control). This trend of reduction in mean estradiol level in all the treatments was observed on 9th day in Group-II ($t_{(18)}$=8.23; P<0.0001), Group-III ($t_{(18)}$=8.73; P<0.0001) and Group-IV ($t_{(18)}$=8.39; P<0.0001) compared to Group-I. On 12th day of experiment highly significant reduction remained in Group-II ($t_{(18)}$=10.39; P<0.0001), Group-III ($t_{(18)}$=9.22; P<0.0001) and Group-IV ($t_{(18)}$=9.90; P<0.0001) compared to Group-I (control). Comparison for estradiol plasma levels among treatment groups for day 3rd, 6, 9 and 12 showed no statistical difference.

Linear regression analysis of variance showed that there was no significant change in plasma estradiol concentration with the advance in days of treatment (b=-1.19±2.48; $F_{(1,3)}$=0.23; P=0.66) in control (Group-I), whereas there was highly significant decrease in plasma estradiol concentration in Group-II (off fed) (b=-33.17±6.26; $F_{(1,3)}$=24.04; P=0.013), as well as Zn treated Group-III (25,000ppm/Kg feed) (b=-37.14±6.86; $F_{(1,3)}$=29.28; P=0.01) and Group-IV (30,000ppm/Kg feed) (b=-33.42±5.01; $F_{(1,3)}$=44.49; P=0.006) (Fig. 5).

Plasma Progesterone Level

The mean plasma concentration of progesterone in the different treatments is shown in Table 7. A significant decrease was observed on 3rd day of experiment in mean progesterone concentration in Group-II ($t_{(8)}$=2.60; P=0.02) compared to control, while Group-III ($t_{(8)}$=1.76; P=0.10) and Group-IV ($t_{(8)}$=1.81; P=0.09) showed no change. On day six (6) highly significant decrease was observed in mean plasma progesterone level in all the treatment groups i.e. Group-II ($t_{(18)}$=19.30; P<0.0001), Group-III ($t_{(18)}$=15.78; P<0.0001) and Group-IV ($t_{(18)}$=20.27; P<0.0001) compared to control. Plasma level of progesterone was also highly affected in both applied methods on 9th and 12th day and showed a significant decrease in Group-II ($t_{(18)}$=16.63; P<0.0001) ($t_{(18)}$=16.51; P<0.0001), Group-III ($t_{(18)}$=16.14; P<0.0001) ($t_{(18)}$=11.89; P<0.0001) and Group-IV ($t_{(18)}$=15.44; P<0.0001) ($t_{(18)}$=12.68; P<0.0001) compared to control. Comparison of plasma progesterone levels within all treatment groups revealed no significant difference on 3rd, 6th, 9th and 12th day of experiment.

Linear regression analysis of variance showed that there was no significant change in plasma progesterone concentration with the advance in days of treatment (b=-0.07±0.11; $F_{(1,3)}$=0.30; P=0.62) in control (Group-I), whereas there was highly significant decrease in plasma progesterone concentration in Group-II (off fed) (b=-0.65±0.21; $F_{(1,3)}$=9.16; P=0.06), as well as Zn treated Group-III (25,000ppm/Kg feed) (b=-0.67±0.20; $F_{(1,3)}$=10.89; P=0.046) and Group-IV (30,000ppm/Kg feed) (b=-0.64±0.19; $F_{(1,3)}$=10.50; P=0.05) with the advance in the days of treatment (Fig. 6).

Plasma Corticosterone Level

The mean plasma concentration of corticosterone in the different treatments is shown in Table 8. On 3rd day of dosage treatment mean corticosterone level started increasing significantly in Group-II ($t_{(18)}$=4.34; P=0.0004), Group-III ($t_{(18)}$=4.24; P=0.001) and Group-IV ($t_{(18)}$=11.89; P<0.0001) compared to Group-I (control) but the elevation was more evident in Group-IV (zinc 30,000ppm). Analysis of plasma corticosterone level on 6th day also revealed highly significant increase in Group-II ($t_{(18)}$=4.59; P=0.0002), Group-III ($t_{(18)}$=8.05; P<0.0001) and Group-IV ($t_{(18)}$=8.21; P<0.0001) compared to Group-I (control). On 9th day of experiment mean corticosterone concentration of Group-II ($t_{(18)}$=17.54; P<0.0001), Group-III

($t_{(18)}$=25.75; P<0.0001) and Group-IV ($t_{(18)}$=20.77; P<0.0001) showed highly significant increase compared to Group-I (control). Highly significant increase in corticosterone level was also observed on 12th day of experiment in Group-II ($t_{(18)}$=33.44; P<0.0001), Group-III ($t_{(18)}$=27.63; P<0.0001) and Group-IV ($t_{(18)}$=27.44; P<0.0001) compared to Group-I (control). Comparison of mean corticosterone concentration showed no significant difference among all treatments throughout the experiment starting from day 1-12.

Linear regression analysis of variance showed that there was no significant change in plasma corticosterone concentration with the advance in days of treatment (b=-0.016±0.16; $F_{(1,3)}$=0.009; P=0.93) was observed in control (Group-I), but highly significant increase in plasma concentration in Group-II (off fed) (b=9.72±0.37; $F_{(1,3)}$=681.2; P=0.0001), Group-III (25,000ppm/Kg feed) (b=9.58±0.57; $F_{(1,3)}$=283.20; P=0.0005) and Group-IV (30,000ppm/Kg feed) (b=8.87±0.83; $F_{(1,3)}$=112.80; P=0.002) with the advance in the days of treatment (Fig. 7).

6 MORPHOMETRY

Mean Ovarian Yolky Follicles Numbers

In Batch-I mean numbers of yolky follicles were categorized according to their diameter and are shown in Table 9. Compared to controls there was highly significant reduction ($P<0.001$) in the mean number of yolky follicles in off fed (Group-II), 25,000ppm zinc/Kg feed (Group-III) and 30,000ppm zinc/Kg feed treatment (Group-IV) in 1-5mm category. Group-III (25,000ppm zinc/Kg feed group) Vs Group-IV (30,000ppm zinc/Kg feed) did not show significant difference. One way analysis of variance also showed highly significant differences in means of different groups $F_{(3,16)}=22.70$; $P<0.001$.

There was no significant difference in mean number of yolky follicles compared to controls in Group-II, Group-III and Group-IV in 5.1-10mm category. In category 10.1-15mm only one yolky follicle was recorded in Group-II and two follicle in Group-IV. In category 15.1-20mm one yolky follicle in Group-III and Group-IV was observed. In categories 20.1-25mm and 25.1-30mm no yolky follicle was seen in all treatment groups.

Mean Ovarian Yolky Follicles Diameter

In Batch-I mean size of yolky follicles was recorded and classified according to their diameter in each category given in Table 10. In yolky follicular diameter category 1-5mm Group-II ($P<0.001$), Group-III ($P<0.05$) and Group-IV ($P<0.05$) showed highly significant reduction in mean diameter compared to Group-I (control) due to off fed conditions, low zinc dose and high zinc dose. There was no significant difference in yolky follicular diameter in category 5.1-10mm in all treatment groups compared to control. In other categories where one or two yolky follicles were observed, their individual diameter was given in the respective table.

Mean Non Yolky Ovarian Follicular Diameter

Ovarian non yolky follicular diameter was arranged in different categories in Table 11. In the Group-II the smallest follicular diameter (≤200µm) reduced highly significantly compared to Group-I ($t_{(150)}$=2.60; P=0.01). Treatment with low and high zinc dose showed non significant difference in follicular diameter compared to that of control. However, in follicular diameter category 201-400µm Group-II showed highly significant reduction in follicular diameter compared to that of Group-I ($t_{(212)}$=5.34; P<0.0001).as well as to that of low zinc dose Group-III ($t_{(111)}$=2.77; P=0.007) and high zinc dose Group-IV ($t_{(164)}$=3.02; P=0.003). In follicular diameter category 601-800µm, Group-II ($t_{(68)}$=4.30; P<0.0001) and Group-IV ($t_{(31)}$=3.84; P=0.001) showed highly significant reduction in diameter compared to control whereas Group-III had significantly higher follicular diameter than Group-II ($t_{(68)}$=3.13; P=0.003) and Group-IV ($t_{(31)}$=2.85; P=0.008) in the same category. In follicular categories 801-1000µm and >1001µm statistical analysis can not be made due to absence of ovarian follicles of this diameter in Group-II and Group-IV whereas significant reduction in follicular diameter in category 801-1000µm and non significant reduction in category >1001µm was found in Group-III compared to Group-I.

Mean Non Yolky Ovarian Oocyte Diameter

Mean diameter of oocytes of non yolky follicles at the end of treatment period are given in Table 12. The lowest category ranged from ≤200µm and the highest category of mean oocyte diameter was >1001µm. The effect of treatment on mean oocyte diameter was observed in all categories of oocyte diameter. In the lowest category of oocyte diameter the highest reduction in diameter was seen in Group-II ($t_{(149)}$=3.08; P=0.003) and Group-IV ($t_{(251)}$=2.60; P=0.001) compared to Group-I. Mean oocyte diameter in Group-II and Group-IV also decreased ($t_{(124)}$=2.39; P=0.019; $t_{(226)}$=2.02; P=0.05 respectively) significantly as compared to Group-III in this category.

In Group-II oocyte diameter showed reduction ($t_{(211)}$=2.85; P=0.005) compared to Group-I in category 201-400µm. Mean oocyte diameter of Group-II in this category also decreased ($t_{(111)}$=2.09; P=0.04) significantly compared to Group-III. There was significant decrease ($t_{(132)}$=2.07; P=0.04) in mean oocyte diameter in category 401-600µm of Group-II compared to that of Group-I. Mean oocyte diameter in this

category of Group-II was also significantly lower compared to that of Group-III ($t_{(105)}$=3.12; P=0.002) and Group-IV ($t_{(159)}$=2.79; P=0.006).

The oocyte diameter in highest category 601-800µm showed highly significant reduction ($t_{(74)}$= 8.71; P<0.0001; $t_{(24)}$=3.70; P=0.001; $t_{(37)}$=5.36; P<0.0001 respectively) in Group-II, Group-III and Group-IV compared to that of Group-I. Mean oocyte diameter in this category of Group-II was also significantly lower compared to that of Group-III ($t_{(68)}$=2.62; P=0.01) and Group-IV ($t_{(81)}$=2.91; P=0.005).

The oocyte diameter of categories 801-1000µm and >1001µm were not observed in Group-II and Group-IV while, oocyte diameter category 801-1000µm decreased significantly ($t_{(17)}$=2.47; P=0.02) in Group-III compared to Group-I.

Mean Non Yolky Follicular Wall Thickness

Mean non yolky follicular wall thickness in the four groups is given Table 13. Mean follicular thickness was divided into different categories on the same basis as was done in the case of follicular and oocyte diameter. Compared to control (Group-I), Group-II ($t_{(149)}$=10.01; P<0.0001) and Group-IV ($t_{(251)}$=11.27; P<0.0001) showed highly significant reduction in mean thickness of follicular wall in category (1-20µm). Group-II compared to Group-III ($t_{(124)}$=5.92; P<0.0001) and Group-IV ($t_{(230)}$=9.02; P<0.0001) showed highly significant reduction in follicular wall thickness in same category (1-20µm). Group-IV **vs** Group-III showed significant reduction ($t_{(226)}$=4.03; P<0.0001) in mean follicular wall thickness. Follicular wall thickness in category 21-40µm showed highly significant reduction in thickness in Group-II ($t_{(212)}$=9.73; P<0.0001) and Group-IV ($t_{(210)}$=4.49; P<0.0001) compared to Group-I (control). Group-II compared to Group-III ($t_{(111)}$=4.15; P<0.0001) and Group-IV ($t_{(164)}$=3.42; P=0.0008) showed highly significant reduction in 21-40µm category of follicular wall thickness.

In follicular wall thickness category 41-60µm, compared to control, there was significant reduction in follicular wall thickness in Group-II ($t_{(132)}$=11.43; P<0.0001), Group-III ($t_{(69)}$=3.68; P=0.0005) and Group-IV ($t_{(123)}$=9.97; P<0.0001). Similarly, Group-II **vs** Group-III ($t_{(105)}$=4.62; P<0.0001) and Group-IV vs Group-III ($t_{(96)}$=3.88; P=0.0002) showed significant reduction in mean follicular wall thickness in this category 41-60µm. In follicular wall thickness category 61-80µm Group-II compared to Group-I showed significant reduction in mean follicular wall thickness ($t_{(74)}$=4.79; P<0.0001). Follicular wall thickness in Group-II also reduced significantly ($t_{(68)}$=3.63; P=0.0006; $t_{(81)}$=4.02; P=0.0001)compared to Group-III and Group-IV. The results showed that off fed condition has severely affected the follicular wall thickness (reduction) compared to low and high doses of zinc.

Mean Non Yolky Ovarian Follicular Number

Mean number of non yolky follicle in all treatment groups are shown in Table 14. Mean number of ovarian follicles were arranged in categories as was done for mean oocyte diameter. In the category ≤200µm mean numbers of ovarian follicles in Group-II and Group-IV ($t_{(8)}$=5.72; P=0.0004; $t_{(8)}$=5.75; P=0.0004) highly significantly

reduced compared to control but decrease in Group-III (low zinc dose) was non significant. Highly significant reduction in mean ovarian follicle number was also seen in Group-II and Group-IV ($t_{(8)}$=4.82; P=0.001; $t_{(8)}$=4.81; P=0.001) in ≤200µm category compared to Group-III which was more evident in Group-II (off fed). Group-II also showed significant decrease in mean ovarian follicle number ($t_{(8)}$=3.94; P=0.004) in category 201-400µm compared to control but Group-III and Group-IV did not show significant differences. There was no significant difference in mean number of ovarian follicles in category 401-600µm of all the three treatment groups compared to control. Follicles of categories 601-800µm, 801-1000µm and >1001µm diameter were present in Group-I but these categories of follicles did not appear in Group-II and Group-IV while, follicles of diameter category 601-800µm appeared in Group-III which showed minor difference in mean number compared to control group (Group-I).

7 HISTOMORPHOLOGY

General and Histological Observations of Ovaries

In birds only the left ovary and oviduct are functional. The ovaries were covered by single columnar epithelium, containing many follicles in the cortical zone. Ovarian weight and size in off fed, low and high zinc dose treatment groups decreased during treatment period compared to Group-I (Table 4). After slaughtering the birds their ovaries were removed. Large yolky and small whitish (transparent) yolky follicles were counted and measured (Table 9 and 10). In the ovary of control birds more yolky follicles were observed compared to treated birds ovary. Blood vessels were visible in yolky ovum (singular form of ova) surrounded by zona pellucida and suture line or stigma was also very clear. In treated birds less yolky follicles and smaller whitish follicles were seen. Microscopic study of cross section of middle portion of the ovary of White Leghorn layer birds at 25th week of age showed that the ovary possessed two distinct regions, an outer cortex and inner medulla. The cortex contained follicles of variable size range. Between the follicles abundant compactly arranged stromal tissue was present. The inner medullar stroma was composed of well vascularised and innervated connective tissue with spaces (lacunae), nerves and blood vessels.

The control ovary contained number of healthy non yolky follicles at various developmental stages. Decrease in number of ovarian follicles was observed in Group-II (off fed) and zinc treated Group-III and Group-IV compared to control group (Fig. 8A). In control group follicles of all diameter categories from ≤200µm to >1001µm were present whereas in zinc high dose treatment groups (30,000pmm zinc/Kg feed) and off fed group larger categories ranging from 601-800µm to >1001µm follicles did not develop (Fig. 8 ABCD). The number of small follicles of category range ≤200µm also decreased in off fed (Fig. 8B) and high dose treatment groups (30,000pmm zinc/Kg feed) (Fig. 8D).

In control group, large follicles contained a well defined elliptical nucleus with distinct network of chromatin and clear nuclear membrane. The ooplasm of the follicle was covered with zona pellucida membrane and attached to granulosa layer through cytoplasmic extensions. The granulosa layer of follicle is composed of single layer of cuboidal cells. Above the granulosa layer basal lamina layer of longitudinal

cells separated the granulosa layer from follicular epithelium (thecal layer). Thecal layer was distinguishable into two layers, the theca interna and the theca externa, in control group. There were numerous thecal glands in follicular epithelium for production of steroid hormones for stimulation of folliculogenesis and ovulation processes.

Oocytes of ovarian follicles in the control group possessed a well defined spherical nucleus with distinct network of chromatin and clear nuclear membrane (Fig. 9A). While in off fed group (Fig. 9B) and zinc treated bird groups, the oocyte nucleus was not well defined and nuclear matrix exhibited shirked appearance (Fig. 9CD).

There was no difference in structure of primordial follicles in treated groups compared to control. Control bird contained normal follicular cytoplasm (Fig.10A) whereas in off fed group and both zinc treated groups, ovaries possessed some larger follicles with abnormal oocytes and disrupted ooplasm (Fig. 10BCD). In these oocytes the nucleus also detached from center and became irregular in shape lacking nuclear material (Fig. 10B).

The thin layer of zona pellucida between ooplasm and granulosa cells was disrupted in some follicles of Group-II, Group-III and Group-IV (Fig. 11BCD). The zona pellucida also detached and lacked cytoplasmic processes into granulosa layer.

The granulosa layer of the follicles was composed of cuboidal cells. Granulosa layer and basal lamina in some follicles of off fed and both zinc treated groups were thick, intermingled with each other and no clear demarcation between them (Fig. 12BCD) was visible but in control birds there was clear demarcation between basal lamina and granulosa layer (Fig. 12A).

The thecal layer was distinguishable into two portions, i.e. theca interna and theca externa. Reduction in thickness of follicular epithelium (thecal layer and granulosa layer) was observed. Theca interna and theca externa layer was seen in all categories of follicles in off fed and low and high dose zinc treated groups compared to control (Fig. 13A). However, greater decrease in thickness of follicular epithelial layer was observed in smaller categories of follicles in off fed and high zinc dose groups **(**Fig. 13BCD).

Follicular epithelial layer (theca interna and theca externa**)** of control group was compactly arranged with clear thecal gland in them. Loose arrangement of

tissue was observed in the theca layers (theca interna and theca externa) in all treated groups compared to control group (Fig. 14A). Apart from loose thecal layers, no thecal gland and disruption was observed in off fed group and both zinc treated groups which was more profound in both off fed and high dose of zinc groups, (30,000ppm zinc/Kg feed) (Fig. 14BCD).

Increased number of atretic follicles was seen in off fed (Fig. 15B) group and birds treated with both doses of zinc (Fig. 15CD) whereas in control group, no atretic follicles were observed (Fig. 15A). Many of these atretic follicles showed disintegrated follicular and granulosa wall. The cytoplasm of atretic follicles showed thick accumulation of yolky vacuole mass and hypertrophied granulosa cells.

Stromal tissue in treated groups was loosely arranged and more interstitial spaces were seen in off fed (Fig. 16B) and zinc treated groups (Fig. 16CD) while in Group-I (control) compact and well organized stroma with less or no lacunae was observed (Fig. 16A).

Table 1: Effect of complete restraint feeding and zinc administration on mean body weight (gm) in control and treated group of young White Leghorn (WLH) layer birds

Treatment groups	Group-I	Group-II	Group-III	Group-IV
Initial Body weight	1139.00±51.91	1164.67±43.72	1211.00±54.01	1188.00±58.60
3rd day	1149.00±42.33	1126.00±40.17	1186.50±53.44	1120.00±65.47
6th day	1180.50±44.32	1078.50±36.57	1176.50±55.04	1113.00±69.43
9th day	1231.00±34.40	1049.00±40.10	1152.00±44.04	1072.50±64.90
12th day	1245.00±45.02	1001.00±35.67^{a***b****}	1121.50±40.69^{c*}	1032.50±59.76^{b***}

Values are means±SEM (N=10)
a=Initial weight of group compared to its final weight.
b=Group-I compared to all other groups at 12th day
c=Group-II compared to Group-III and group-IV at 12th day.
d=Group-III compared to Group-IV at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 4: Effect of complete restraint feeding and zinc administration on mean ovarian, oviduct and liver weight (gm), length and width (mm) in young White Leghorn (WLH) layer birds, slaughtered on 12th day of experiment (Batch-I)

Treatment groups	Ovaries			Oviduct		Liver
	Weight	**Length**	**width**	**weight**	**Length**	**Weight**
Group-I	46.36±1.85	35.51±3.91	15.51±1.91	43.69±3.14	59.16±1.70	45.30±3.28
Group-II	5.60±0.42^{a****}	24.71±1.38^{a*}	16.12±0.66	16.57±1.41^{a****}	34.25±2.14^{a****}	23.19±0.67^{a***}
Group-III	11.46±1.66^{a****b***}	24.40±2.64^{a*}	15.56±1.50	14.12±2.16^{a****}	34.00±3.99^{a****}	33.99±4.41^{b*}
Group-IV	8.76±1.33^{a****}	23.86±2.48^{a*}	17.41±2.02	12.81±1.30^{a****}	27.10±1.92^{a****b*}	34.16±1.93^{a**b***}

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 6: Effect of complete restraint feeding and zinc administration on mean plasma estradiol concentration (pg/ml) in young White Leghorn (WLH) layer birds slaughtered on 12th day of experiment (Batch-I)

	Group I	Group-II	Group-III	Group-IV
Initial	214.95±17.65	198.41±20.07	217.70±11.28	196.74±11.63
3rd day	206.52±11.65	120.52±10.23^{a****}	152.51±15.01^{a***}	141.62±15.63^{a***}
6th day	201.47±16.25	105.40±4.99^{a****}	103.05±9.44^{a****}	100.70±4.04^{a****}
9th day	198.61±13.33	73.68±7.27^{a****}	76.40±4.32^{a****}	74.35±6.46^{a****}
12th day	212.91±13.95	56.03±5.78^{a****}	70.05±6.72^{a****}	63.28±5.80^{a****}

Values are means±SEM (N=10)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 7: Effect of complete restraint feeding and zinc administration on mean plasma progesterone concentration (ηg/ml) in young White Leghorn (WLH) layer birds, slaughtered on 12th day of experiment (Batch-I)

	Group I	Group-II	Group-III	Group-IV
Initial	4.56±0.62	4.49±0.31	4.54±0.44	4.44±0.57
3rd day	4.06±0.41	2.60±0.39^{a**}	2.98±0.46	2.90±0.50
6th day	4.84±0.11	1.91±0.10^{a****}	1.86±0.15^{a****}	1.93±0.09^{a****}
9th day	4.91±0.16	1.77±0.11^{a****}	1.92±0.10^{a****}	1.75±0.13^{a****}
12th day	4.46±0.14	1.68±0.10^{a****}	1.74±0.18^{a****}	1.82±0.16^{a****}

Values are means±SEM (N=10)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 8: Effect of complete restraint feeding and zinc administration on mean plasma corticosterone concentration (ηg/ml) in young White Leghorn (WLH) layer birds, slaughtered on 12th day of experiment (Batch-I)

	Group I	Group-II	Group-III	Group-IV
Initial	8.52±0.97	8.16±0.72	7.84±0.43	7.47±0.51
3rd day	7.41±0.72	17.48±2.20^{a****}	20.85±3.09^{a****}	22.43±2.52^{a****}
6th day	8.04±0.84	28.66±4.41^{a****}	26.47±2.13^{a****}	27.11±2.1^{a****}
9th day	7.69±0.90	35.73±1.32^{a****}	37.07±0.70^{a****}	35.34±0.98^{a****}
12th day	8.30±0.82	47.65±0.85^{a****}	47.63±1.16^{a****}	45.34±1.07^{a****}

Values are means±SEM (N=10)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 9: Effect of complete restraint feeding and zinc administration on mean number in different category range (mm) of ovarian yolky follicles in White Leghorn layer birds at 25th week of age slaughtered on 12th day of Experiment (Batch-I)

	Yolky Follicle Number Category Range (mm)					
	1-5mm	**5.1-10mm**	**10.1 -15mm**	**15.1-20mm**	**20.1-25mm**	**25.1-30mm**
Group I	90.60±11.46	2.00±0.55	1.20±0.37	1.40±0.51	1.20±0.49	1.80±0.66
Group II	25.80±1.77[a***]	1.75±0.43	(1)	-	-	-
Group III	38.40±3.47[a***]	2.50±0.77	0.80±0.37	(1)	-	-
Group IV	33.80±2.48[a***]	1.00±0.37	(2)	(1)	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001
Digits in parenthesis are number of yolky follicle in respective group

Table 10: Effect of complete restraint feeding and zinc administration on mean diameter (mm) category range of ovarian yolky follicles in White Leghorn layer birds at 25th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Yolky Follicles Diameter Category Range (mm)					
	1-5mm	**5.1-10mm**	**10.1 -15mm**	**15.1-20mm**	**20.1-25mm**	**25.1-30mm**
Group I	4.37±0.19	7.43±0.77	13.47±0.48	18.31±0.59	22.84±0.39	26.656±0.74
Group II	3.60±0.40[a***]	6.38±0.48	11.36 (1)	-	-	-
Group III	3.81±0.34[a*]	7.15±0.43	11.61±0.18	17.46 (1)	-	-
Group IV	3.85±0.31[a*]	6.84±0.46	12.13 (2)	15.36 (1)	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001
Digits in parenthesis are number of yolky follicle in respective group

Table 11: Effect of complete restraint feeding and zinc administration on mean non yolky follicular diameter (µm) in White Leghorn layer birds at 25th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Mean Follicular Diameter Categories (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group I	143.92±3.99	322.20±4.79	524.72±9.71	730.42±19.23	920.94±16.59	1514.29±105.48
Group II	129.44±3.59^{a***}	282.79±5.61^{a****}	514.24±5.57	664.32±5.43^{a****}	-	-
Group III	140.08±5.58	317.91±12.27^{b***}	519.99±12.43	715.81±24.26^{b***}	868.20±18.17^{a*}	1283.82±85.81
Group IV	137.90±3.93	308.51±6.45^{b***}	505.44±6.28	650.62±10.91^{a****c***}	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05**=0.02***=0.01 and ****=0.001

Table 12: Effect of complete restraint feeding and zinc administration on mean non yolky oocyte diameter (µm) in White Leghorn layer birds at 25th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Mean Oocytes Diameter Categories (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group I	120.57±3.85	284.04±5.19	473.97±9.67	711.40±12.46	877.09±22.95	1353.57±91.60
Group II	105.17±2.66^{a***}	259.04±6.66^{a***}	450.53±5.50^{a*}	584.03±6.78^{a****}	-	-
Group III	119.62±5.57^{b*}	290.99±12.44^{b**}	489.00±11.57^{b***}	631.73±18.59^{a****b***}	765.63±25.39^{a**}	1119.70±92.14
Group IV	107.50±3.51^{a***c*}	276.20±6.41	473.76±6.28^{b***}	621.52±10.97^{a****b***}	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05**=0.02***=0.01 and ****=0.001

Table 13: Effect of complete restraint feeding and zinc administration on mean non yolky follicular wall thickness (µm) in White Leghorn layer birds at 25th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Mean Follicular Wall Thickness Categories (µm)					
	≤20µm	**21-40µm**	**41-60µm**	**61-80µm**	**81-100µm**	**>101µm**
Group I	17.02±0.22	32.27±0.39	54.27±0.59	71.75±1.31	93.03±3.74	131.41±14.71
Group II	13.37±0.22^{a****}	25.84±0.66^{a****}	44.58±0.54^{a****}	65.11±1.15^{a****}	-	-
Group III	16.22±0.43^{b****}	30.89±1.06^{b****}	50.06±1.04^{ab****}	71.38±2.00^{b***}	89.90±1.49	119.98±5.01
Group IV	15.16±0.06^{ab****c***}	28.80±0.59^{ab****}	45.01±0.63^{ac****}	69.91±0.66^{b****}	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table14: Effect of complete restraint feeding and zinc administration on mean number of non yolky follicles per section of ovary in White Leghorn layer birds at 25th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Mean Follicle Number Category Range (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group-I	27.00±2.17	8.00±0.95	5.40±1.75	2.60±0.98	1.25±0.22	2.00±0.45
Group-II	9.60±2.14^{a****}	3.40±0.68^{a****}	3.00±1.05	-	-	-
Group-III	22.80±1.71^{b***}	7.00±2.17	5.20±1.66	2.20±0.58	-	-
Group-IV	12.00±1.92^{ac****}	4.80±1.28	1.80±0.37	-	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

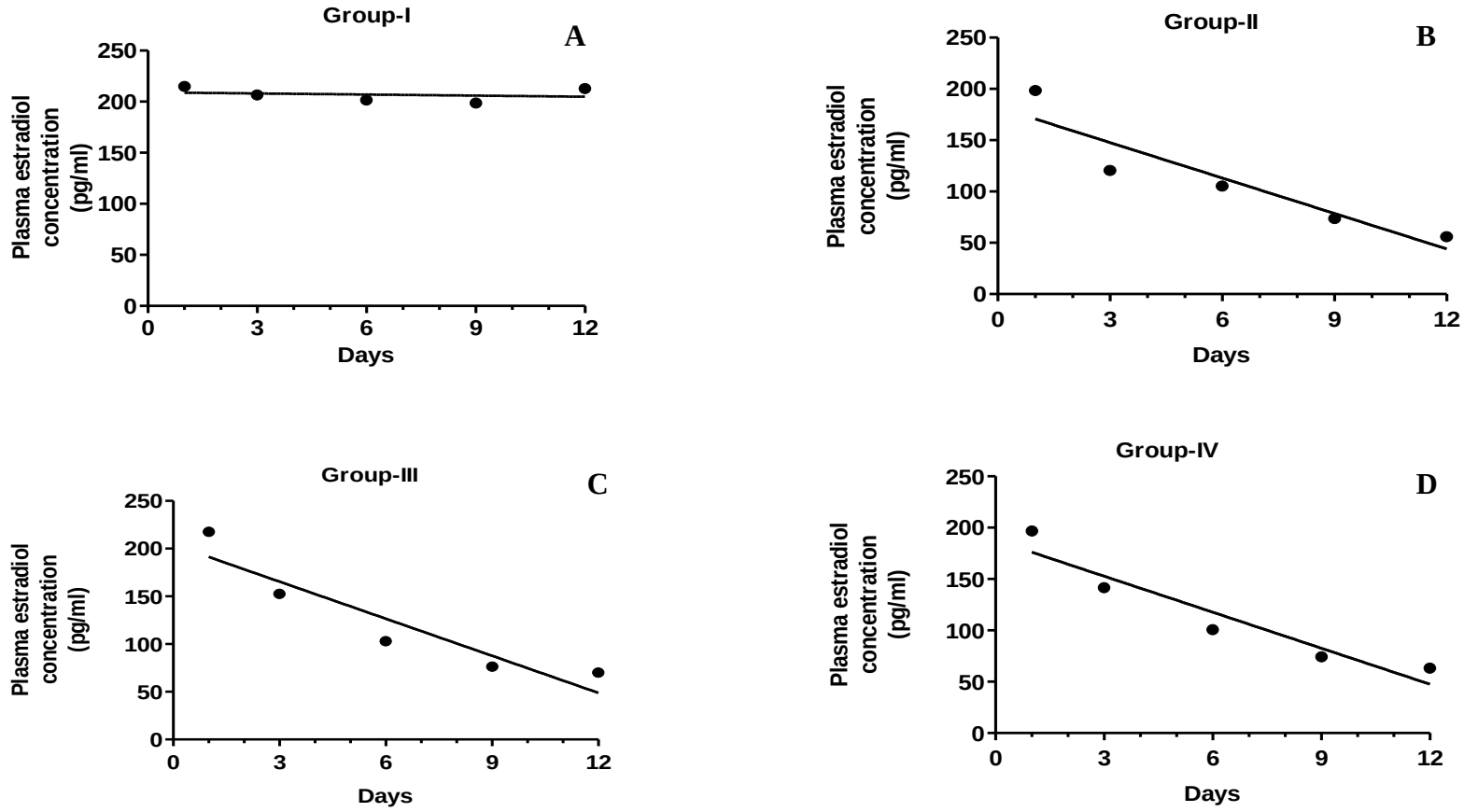


Fig. 5: Regression line showing significant decrease in mean plasma estradiol levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during twelve days of treatment in White Leghorn layer birds at 25th week of age (Batch-I).

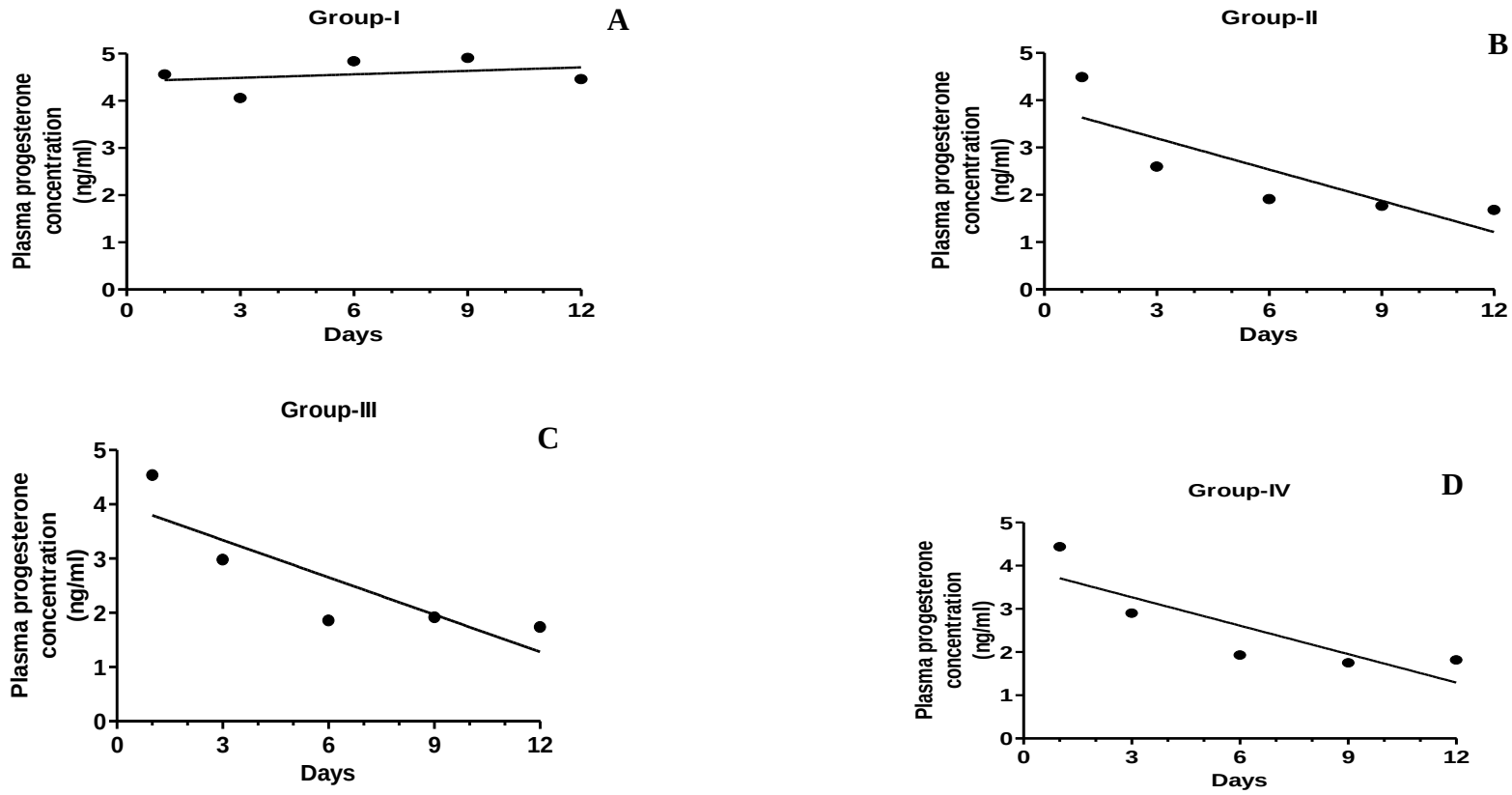


Fig. 6: Regression line showing significant decrease in mean plasma Progesterone levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during twelve days of treatment in White Leghorn layer birds at 25th week of age (Batch-I).

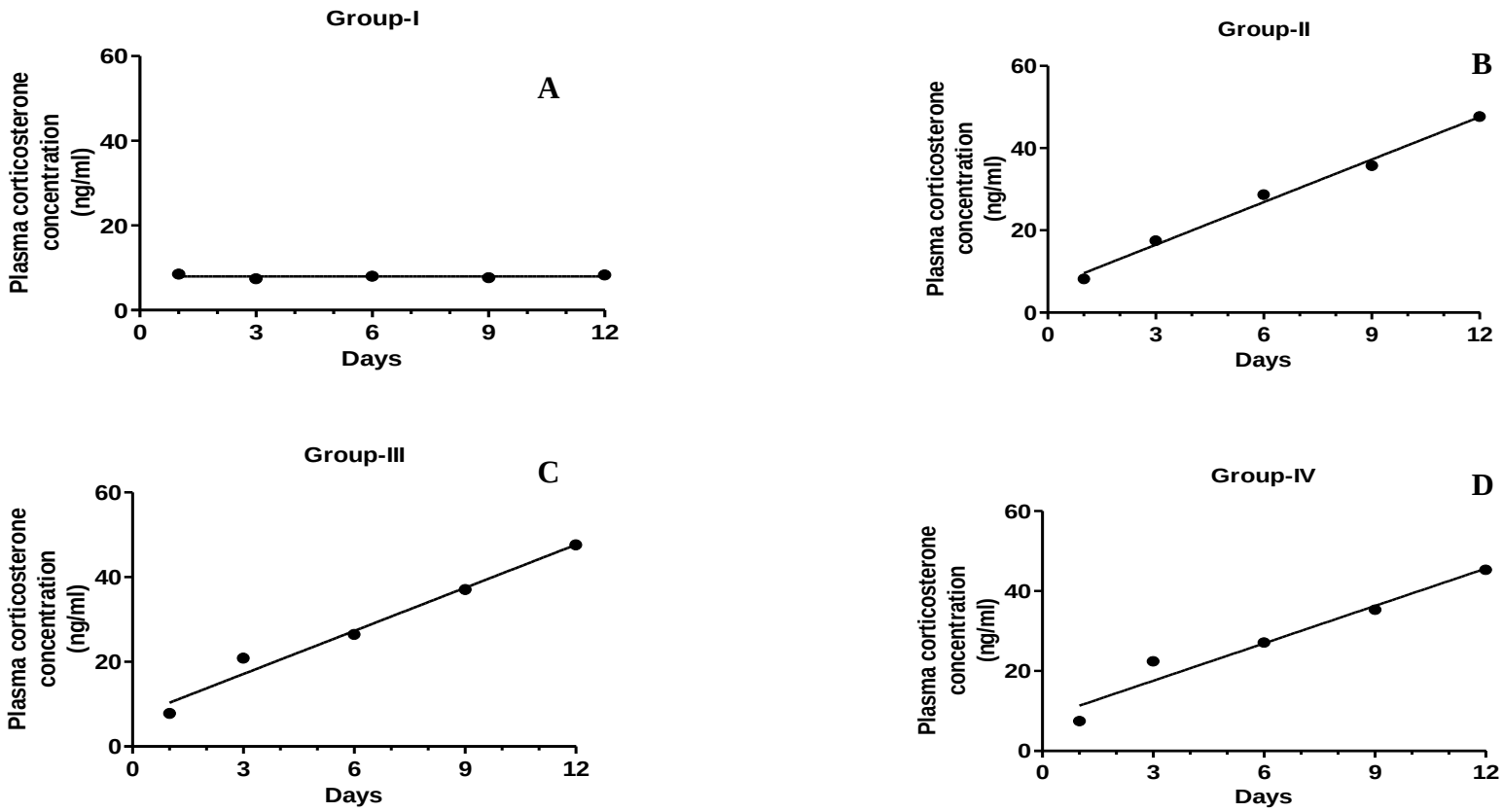


Fig. 7: Regression line showing significant increase in mean plasma corticosterone levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during twelve days of treatment in White Leghorn layer birds at 25th week of age (Batch-I).

BATCH-II

1. BODY WEIGHT

Body weight in each group was measured at an interval of two days and is given in Table 15. At 15th day of experiment mean body weight of all treatment groups was also compared with control (Group-I) and among all groups (Fig. 17). Mean body weight in each group at 15th day of experiment was compared with mean body weight at 3rd day after withdrawal of treatment (Fig. 18).

Group-I (Control Group)

Birds in this group were kept on normal feeding regime throughout the experiment. A significant increase ($t_{(8)}$=2.93; P=0.02) in mean weight of Batch II birds was observed at end of experiment compared to mean body weight at day 15 of the experiment. The regression analysis of variance indicates a gradual highly significant increase in body weight on 27th day (b=28.14 ± 2.86; $F_{(1,3)}$= 96.70; P=0.002), this increase in body weight was over the period of 15-days..

Group-II (Off Fed)

The remaining five birds in this group (Batch-II) were maintained on normal feed for 15-days. In Batch-II mean body was 1154±34.58gm at the start and 1272±20.68gm at end of experiment, there was highly significant ($t_{(8)}$=5.09; P=0.001) increase in mean weight. The linear regression analysis indicates significant increase in mean body weight (b=58.20±11.40; $F_{(1,3)}$=26.05; P=0.015) over the period of fifteen days.

Group-III (25,000ppm Zinc/Kg Feed)

After withdrawal of treatment the birds in this batch like other groups were given normal feed and slaughtered after 27-days, showed non significant increase in mean body weight at the end of experiment ($t_{(8)}$=1.65; P=0.14) compared to mean body weight of the birds at 15th day. The regression analysis of variance indicates highly significant gain in body weight (b=34.70±7.324; $F_{(1,3)}$=22.45; P=0.02) on 15th day after withdrawal of treatment.

Group-IV (30,000ppm Zinc/Kg Feed)

The remaining five birds in this group were reverted to normal feed for another 15-day. In this Batch non-significant gradual increase ($t_{(8)}$=1.34; P=0.22) in final mean body weight was observed at the end of the experiment compared to mean body weight at day fifteen (15) of experiment. The regression analysis of variance indicates highly significant increase (b=42.40±6.04; $F_{(1,3)}$=49.32; P=0.006) in body weight over 15-days period.

Comparisons

During the whole period after the withdrawal of treatment, the birds belonging to control group and treated group grew very well in term of weight and gradually the mean body weight gain in all groups was more or less similar to control group (Group-I). In Group-II, there was significant increase ($t_{(8)}$=2.59; P=0.03) in mean body weight but Group-III ($t_{(8)}$=1.81; P=0.11) and Group-IV ($t_{(8)}$=1.36; P=0.21) showed non significant increase in mean body weight gain as compared to Group-I during this period due to administration of normal feed. The mean body weight of Group-III ($t_{(8)}$=0.51; P=0.62) and Group-IV ($t_{(8)}$=0.19; P=0.85) did not differ significantly as compared to Group-II. Group-III vs Group-IV also showed non significant ($t_{(8)}$=0.22; P=0.83) change in their body weight.

Table 15: Changes in mean body weight (gm) in young White Leghorn (WLH) layer birds at 25th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

Treatment groups	Group-I	Group-II	Group-III	Group-IV
3rd day	1154.00±34.58	1147.00±29.14	1281.00±29.09	1225.00±91.98
6th day	1198.00±21.37	1241.00±43.26	1338.00±45.10	1297.00±105.46
9th day	1205.00±29.50	1334.00±47.81	1399.00±63.49	1339.00±95.03
12th day	1243.00±20.10	1365.00±51.19	1407.00±79.37	1383.00±95.89
15th day	1272.20±20.68^{a**}	1376.00±34.33^{a****b*}	1420.00±78.87	1394.00±86.93

Values are means±SEM (N=5)
a=Initial weight of that group compared to final weight.
b=Group-I compared to all other groups at 15th day
c=Group-II compared to Group-III and Group-IV at 15th day
d=Group-III compared to Group-IV at 15th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

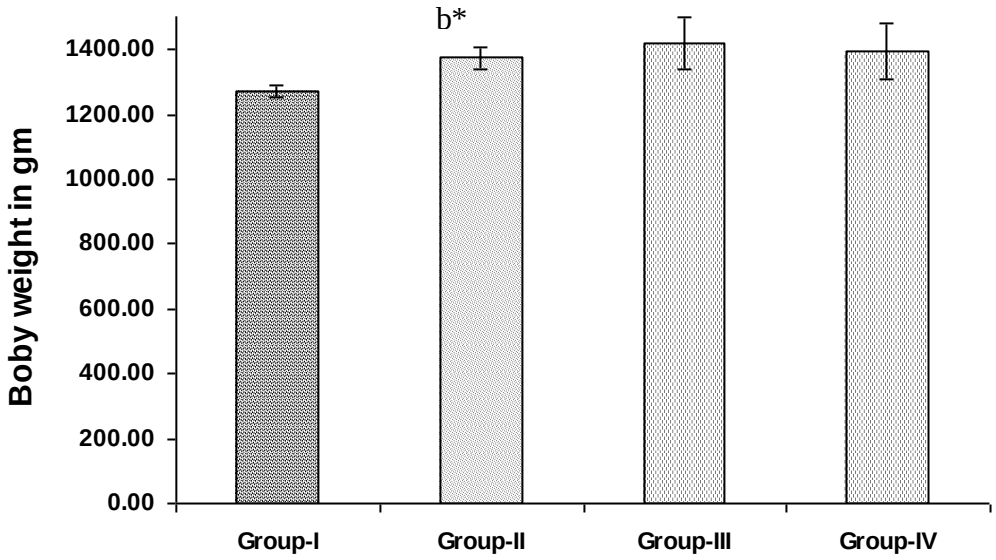


Fig.17. Effect of withdrawal of restraint feeding and zinc administration on mean body weight (gm) in control and treated groups of young White Leghorn (WLH) layer birds on 15th day of experiment (Batch-II).
b=Group-II compared to Group-III and Group-IV at 15th day
c=Group-III compared to Group-IV at 15th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

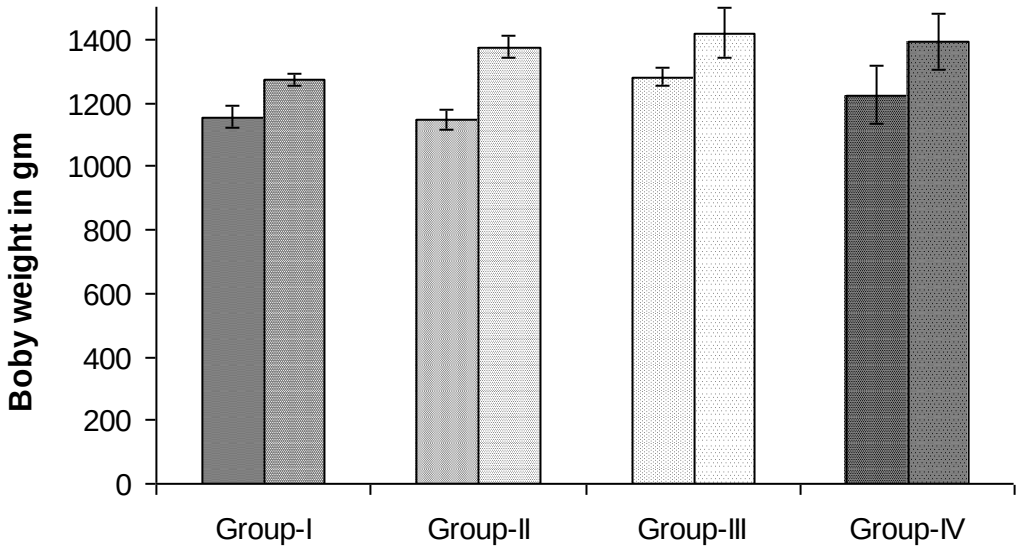


Fig 18: Comparison of initial body weight and body weight (gm) on 15th day of restraint feeding and zinc administration in control and treated groups of young White Leghorn (WLH) layer birds (Batch-II).
a=Group-I compared to all other groups at 15th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

2. SECONDARY SEXUAL CHARACTERISTICS

Combs and Wattles

Secondary sexual characters of the birds like comb and wattles were also examined in control and treated groups (Table 16).

Comb

There was no significant difference in mean comb length ($t_{(8)}$=0.39; P=0.71), ($t_{(8)}$=0.26; P=0.80) and width ($t_{(8)}$=1.62; P=0.14), ($t_{(8)}$=0.17; P=0.87) in Group-II and Group-IV but a significant increase in comb length ($t_{(8)}$=3.89; P=0.005) and width ($t_{(8)}$=2.86; P=0.02) was observed in Group-III compared to Group-I (Control). Similarly, a significant increase in comb length ($t_{(8)}$=2.43; P=0.04) and non significant difference in comb width ($t_{(8)}$=0.36; P=0.73) was observed in birds of Group-III compared to Group-II (off fed). Group-II Vs Group-IV also showed no significant change in comb length ($t_{(8)}$=0.06; P=0.96) and width ($t_{(8)}$=1.09; P=0.31). Group-III Vs Group-IV showed non-significant decrease in mean comb length ($t_{(8)}$=1.15; P=0.29) and comb width ($t_{(8)}$=1.44; P=0.19).

Wattles

In Batch-II which was slaughtered after 27 days, a non significant difference was noticed in mean wattles length in Group II ($t_{(8)}$=1.26; P=0.24) Group-III ($t_{(8)}$=2.14; P=0.064) and Group-IV ($t_{(8)}$=0.34; P=0.75) as compared to Group-I (control). Similarly, wattles length in Group-IV ($t_{(8)}$=1.04; P=0.33) increased non significantly whereas Group-III ($t_{(8)}$=2.90; P=0.02) showed significant increase as compared to Group-II (off fed). Wattles length in Group-III vs Group-IV ($t_{(8)}$=1.08; P=0.31) also remained unchanged.

Wattles width increased significantly in Group-III ($t_{(8)}$=2.47; P=0.04) but difference in Group-II ($t_{(8)}$=2.06; P=0.07) and Group-IV ($t_{(8)}$=0.05; P=0.96) was non significant as compared Group-I. The comparison of Group-II vs Group-III ($t_{(8)}$=0.10; P=0.92) and Group-IV ($t_{(8)}$= 1.95; P=0.09) showed non significant change. Wattles width of Group-III ($t_{(8)}$=0.10; P=0.92) and Group-IV ($t_{(8)}$=1.95; P=0.09) was also not significantly different compared to Group-II (off fed). While Group-III Vs Group-IV comparison showed significant ($t_{(8)}$=2.32; P=0.05) difference in wattles width.

Table 16: Changes in mean comb and wattles length and width (mm) in young White Leghorn (WLH) layer birds slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

Treatment groups	Comb		Wattles	
	Length	Width	Length	Width
Group-I	72.24±1.88	38.59±0.69	28.19±0.72	23.42±1.47
Group-II	73.56±2.85	41.83±1.87	26.64±0.99	27.90±1.74
Group-III	$81.16\pm1.31^{ab*}$	$42.62\pm1.23^{a*}$	$31.29\pm1.26^{b**}$	$28.12\pm1.39^{a*}$
Group-IV	73.94±6.16	38.07±2.90	28.86±1.88	$23.52\pm1.43^{c*}$

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

3 EFFECT ON OVARY, OVIDUCT AND LIVER

Mean ovarian weight, length and width, oviduct length and weight and liver weight of control and all treated groups in Batch-II were recorded in Table 17.

There was no significant change in mean ovarian weight, length and width of Batch II in Group-II ($t_{(8)}$=0.63; P=0.54), ($t_{(8)}$=0.34; P=0.74), Group-III ($t_{(8)}$=0.16; P=0.88), ($t_{(8)}$= 0.17; P=0.87) and Group-IV ($t_{(8)}$=0.07; P=0.94), ($t_{(8)}$=0.39; P=0.70) compared to Group-I (control). Group-III and Group-IV also showed no significant difference in mean ovarian weight, length and width compared to Group-II (off fed). Same trend was observed in Group-III Vs Group-IV comparison.

In 27-days batch (Batch-II), there was highly significant decrease in mean oviductal weight in Group-II ($t_{(8)}$=4.65; P=0.002), Group-III ($t_{(8)}$=3.56; P=0.007) and Group-IV ($t_{(8)}$=5.27; P=0.0008) compared to Group-I (control). There was no appreciable difference in oviductal weight in Group-III ($t_{(8)}$=1.15; P=0.28) and Group-IV ($t_{(8)}$= 0.79; P=0.45) Vs Group-II (Off fed). Same trend was observed in oviductal weight ($t_{(8)}$= 1.91; P=0.09) Group-III Vs Group-IV comparison.

In 27-days batch (Batch-II), mean oviductal length reduced significantly in Group-II ($t_{(8)}$=2.88; P=0.02) and Group-IV ($t_{(8)}$=2.48; P=0.04) but decreased in Group-III ($t_{(8)}$=1.80; P=0.11) was non significant compared to Group-I (control). The comparison between Group-III ($t_{(8)}$=1.32; P=0.22) and Group-IV ($t_{(8)}$=0.96; P=0.36) Vs Group-II also showed non significant differences. In Group-III Vs Group-IV ($t_{(8)}$= 0.46; P=0.66) no significant difference was observed.

Mean liver weight decreased significantly in Group-II ($t_{(8)}$=10.32; P<0.0001), Group-III ($t_{(8)}$=6.87; P=0.0001) and Group-IV ($t_{(8)}$=8.28; P<0.0001) compared to Group-I (control). Mean weight of liver decreased non-significantly in Group-II compared to Group-III ($t_{(8)}$=2.20; P=0.059) and Group-IV ($t_{(8)}$=2.16; P=0.06). In Group-III Vs Group-IV ($t_{(8)}$=0.36; P=0.73) no significant change was seen.

4. ZINC DEPOSITION IN DIFFERENT ORGANS TISSUE

Zinc deposition (µg/gm) in ovary, liver and kidney was measured in all groups of Bath-II which is presented in Table 18.

Deposition Of Zinc In Ovary

In Batch-II, there was no appreciable difference in zinc deposition between Group-I and Group-II ($t_{(8)}$=1.28; P=0.24) birds. However, birds treated with 25,000 zinc dosage (Group-III) ($t_{(8)}$=3.95; P=0.004) and those treated with 30,000ppm dosage (Group-IV) ($t_{(8)}$=9.02; P<0.0001) showed significantly higher zinc deposition compared to Group-I (control). No significant difference was found in mean zinc deposition in Group-II vs Group-III ($t_{(8)}$=2.69; P=0.03) whereas highly significant decrease in Group-II vs Group-IV ($t_{(8)}$=6.89; P=0.0001) was observed. Within treated groups Group-IV showed significant ($t_{(8)}$=3.32; P=0.01) increase in zinc deposition compared to Group-III.

Deposition Of Zinc In Liver

In this experiment, mean zinc deposition in liver of birds slaughtered after 27-days (Batch-II) was also measured. Group-III (25,000ppm zinc treated) ($t_{(8)}$=9.26; P<0.0001) as well as Group-IV (30,000ppm zinc treated) ($t_{(8)}$=12.04; P<0.0001) showed significantly higher concentration compared to Group-I (control). There was highly significantly increased zinc deposition in 27-days batch (Batch-II) in Group-III (25,000ppm dosage) ($t_{(8)}$=11.61; P<0.001) and Group-IV (30,000ppm dosage) ($t_{(8)}$=14.80; P<0.001) compared to Group-II. The difference in zinc deposition in Group-III vs Group-IV was not significant ($t_{(8)}$=1.16; P=0.28).

Deposition Of Zinc In Kidneys

In Batch-II, like in Batch-I, the zinc deposition in Group-I (control) and Group-II (off fed) was similar. However, birds treated with 25,000 zinc dosage (Group-III) ($t_{(8)}$=4.85; P=0.001) and those treated with 30,000ppm dosage ($t_{(8)}$=4.417; P=0.002) showed significantly higher zinc deposition compared to Group-I (control). In Batch-II there was also significant difference in zinc deposition in Group-III ($t_{(8)}$=4.47; P=0.002) and Group-IV ($t_{(8)}$=3.64; P=0.006) compared to Group-II (off fed). However, in Group-III vs Group-IV a non significant ($t_{(8)}$=1.52; P=0.17) difference was seen.

Table 18: Mean Zinc deposition concentration (µg/g) in ovary, liver and kidney in WLH layer birds at 25th week of age after withdrawal of restraint feed and zinc administration slaughtered on 15th day of experiment (Batch-II)

Treatment groups	Batch-II (slaughtered on 15th day of experiment)		
	Ovary	**Liver**	**Kidney**
Group-I	1.21±0.16	11.34±0.51	2.25±0.18
Group-II	2.50±0.18	12.33±1.44	2.71±0.10
Group-III	$2.48\pm0.17^{a***}$	$41.74\pm3.07^{ab****}$	$3.32\pm0.13^{a****b***}$
Group-IV	$3.74\pm0.33^{ab****c***}$	$46.78\pm2.74^{ab****}$	$3.99\pm0.39^{ab***}$

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

5 HORMONAL ESTIMATION

Hormonal analysis for mean plasma Estradiol, Progesterone and Corticosterone is shown in Tables 19, 20 and 21 respectively.

Plasma Estradiol Level

The mean plasma concentration of estradiol in the different treatments is shown in Table 19. The estradiol levels started to rise in all treated groups after withdrawal of treatment however, plasma estradiol levels remained significantly lower than that of the control in all the groups throughout the experimental period. On day 3 after molt induction, the hens in Group-II ($t_{(8)}$=8.43; P<0.0001), Group-III ($t_{(8)}$=7.40; P <0.0001) and Group-IV ($t_{(8)}$=8.79; P <0.0001) showed lower levels of estradiol in their plasma, compared to the levels in Group-I (control). On day six after withdrawal of treatment plasma levels of estradiol of Group-II ($t_{(8)}$=5.82; P=0.0004), Group-III ($t_{(8)}$=6.09; P=0.0003) and Group-IV ($t_{(8)}$=4.81; P=0.001) were also significantly lower than Group-I (control). The increase in mean estradiol level continued in all treated groups up to 9^{th} and 12^{th} day but was still significantly lower in Group-II ($t_{(8)}$=6.02; P=0.0003), Group-III ($t_{(8)}$=3.99; P=0.004) and Group-IV ($t_{(8)}$=3.50; P=0.008) on 9^{th} day and Group-II ($t_{(8)}$=3.91; P=0.005), Group-III ($t_{(8)}$=3.36; P=0.009) and Group-IV ($t_{(8)}$=2.57; P=0.03) on 12^{th} day of after the withdrawal of treatment compared to Group-I (control). The elevated plasma estradiol levels remained lower in all treatment Group-II ($t_{(8)}$=3.73; P=0.006), Group-III ($t_{(8)}$=3.68; P=0.006) and Group-IV ($t_{(8)}$=3.24; P=0.01) up to end of experiment i.e. on 15^{th} day compared to Group-I (control). There was no significant difference in the estradiol levels among treatment groups when they were compared to each other on days 3, 6, 9, 12 and 15.

Linear regression analysis of variance shows that there is no significant difference in plasma estradiol concentration with the advance in days of treatment withdrawal (b=5.23±1.93; $F_{(1,3)}$=7.32; P=0.07) in control (Group-I), whereas there is highly significant increase in plasma estradiol concentration in Group-II (off fed) (b=20.25±3.21; $F_{(1,3)}$=39.72; P=0.008), Group-III (25,000ppm/Kg feed) (b=23.50±4.29; $F_{(1,3)}$=30.05; P=0.01) and Group-IV (30,000ppm/Kg feed) (b=27.18±3.83; $F_{(1,3)}$=50.34; P=0.005) with the advance in days of treatment withdrawal (Fig. 19).

Plasma Progesterone Level

The mean plasma concentration of progesterone in the different treatments is shown in Table 20. On 3rd day after withdrawal of treatment increase in plasma progesterone levels was observed in all treated groups but was significantly lower in Group-II ($t_{(8)}$=5.64; P=0.0005), Group-III ($t_{(8)}$=6.23; P=0.0003) and Group-IV ($t_{(8)}$=6.57; P=0.0002) compared to Group-I (control). On day six (6) after withdrawal of treatment, there was also highly significant decrease in mean plasma progesterone level in all the treatment groups i.e. Group-II ($t_{(8)}$=4.14; P=0.003), Group-III ($t_{(8)}$=6.49; P=0.0002) and Group-IV ($t_{(8)}$=5.77; P=0.0004) compared to control. On 9th (nine) day after withdrawal of treatment plasma levels of progesterone remained lower in Group-II ($t_{(8)}$=3.19; P=0.01), Group-III ($t_{(8)}$=9.08; P<0.0001) and Group-IV ($t_{(8)}$=3.31; P =0.01) compared to Group-I (control). Comparison of groups on day 12 (twelve) revealed that plasma progesterone concentration was significantly lower in Group-II ($t_{(8)}$=2.89; P=0.02) and Group-III ($t_{(8)}$=2.25;P=0.05) but difference in Group-IV ($t_{(8)}$=2.07; P=0.07) was non significant compared to Group-I (control). Mean plasma progesterone levels in all treated groups reached the same level as in Group-I (control) on 15th day of experiment. Comparison among all treatment groups revealed no significant difference in mean plasma progesterone level on 3, 6, 9, 12 and 15 day of experiment.

Linear regression analysis of variance shows that there is no significant change in plasma progesterone concentration with the advance in days of experiment (b=0.05±0.059; $F_{(1,3)}$=0.76; P=0.45) in control (Group-I), where as there is highly significant increase in progesterone concentration in Group-II (off fed) (25,000ppm/Kg feed) (b=0.41±0.12; $F_{(1,3)}$=11.88; P=0.04), Group-III (b=0.33 ± 0.056; $F_{(1,3)}$=34.08; P=0.01) and Group-IV (30,000ppm/Kg feed) (b=0.41±0.10; $F_{(1,3)}$=16.07; P=0.03) with advance in days after withdrawal of treatment (Fig. 20).

Plasma Corticosterone Level

The mean plasma concentration of corticosterone in the different treatments is shown in Table 21. On 3rd day after withdrawal of treatment mean corticosterone level decreased but remained significantly elevated in Group-II ($t_{(8)}$=15.64; P<0.0001), Group-III ($t_{(8)}$=6.21; P=0.0003) and Group-IV ($t_{(8)}$=14.90; P<0.0001) compared to Group-I (control). Corticosterone level on day 6 after withdrawal of treatment also showed significant increase in Group-II ($t_{(18)}$=6.43; P=0.0002), Group-III ($t_{(8)}$=7.79; P<0.0001) and Group-IV ($t_{(8)}$=10.56; P<0.0001) compared to Group-I (control). On day nine (9) after the withdrawal of treatment mean corticosterone concentration of Group-II ($t_{(8)}$=4.40; P=0.002), Group-III ($t_{(8)}$=6.16; P=0.0003) and Group-IV ($t_{(8)}$=6.86; P=0.0001) was highly significantly greater compared to Group-I (control). A significantly escalated corticosterone level was also observed in Group-II ($t_{(8)}$=2.57; P=0.03), Group-III ($t_{(8)}$=5.09; P=0.0009) and Group-IV ($t_{(8)}$=5.62; P=0.0005) compared to Group-I (control) on 12 day after the withdrawal of treatment and on 15th day Group-II ($t_{(8)}$=4.00; P=0.004), Group-III ($t_{(8)}$=4.29; P=0.003) and Group-IV ($t_{(8)}$=4.72; P=0.002) still showed highly significant increased corticosterone level compared to Group-I (control). Comparison for mean corticosterone levels among treatment groups for day 3, 6, 9, 12 and 15 showed no statistical difference.

Linear regression analysis of variance showed that there was no significant difference in plasma corticosterone concentration with the advancing days towards withdrawal of treatment (b=0.13±0.086; $F_{(1,3)}$=2.10; P=0.24) in control (Group-I), where as there is highly significant decrease in corticosterone concentration in Group-II (b=-5.03±0.55; $F_{(1,3)}$=84.30; P=0.003), Group-III (25,000ppm/Kg) (b=-4.05±0.52; $F_{(1,3)}$=61.07; P=0.004) and Group-IV (30,000ppm/Kg) (b=-4.09±0.93; $F_{(1,3)}$=19.60; P=0.02) with the advance in days towards treatment withdrawal (Fig. 21).

6 MORPHOMETRY

Mean Ovarian Yolky Follicles Numbers

In Batch-II all treatments were withdrawn and chicks in this batch were slaughtered on 15th day of experiment. Chicks were fed on normal feed in all treatment groups. Yolky follicles was recorded in control group and in all treatment groups, presented in Table 22. The highest mean number of yolky follicles was observed in the smallest category of follicles (1-5mm) in all groups. In this category mean number of follicles was significantly low ($P<0.001$) in Group-II (off fed), Group-III (zinc 25,000ppm/Kg feed) and Group-IV (zinc 30,000ppm/Kg feed) compared to control. One way analysis of variance showed highly significant difference in means among different groups ($F_{(3,16)}=36.23$; $P<0.0001$) in this category.

In all other categories of yolky follicles there was no significant difference in mean yolky numbers in all treatment groups compared to control group. The results indicated that reversion to normal feed has improved the growth of yolky follicles in all the categories of follicles.

Mean Ovarian Yolky Follicles Diameter

In Batch-II mean diameter of yolky follicles was arranged in different category ranges according to their size (Table 23). In category 1-5mm there was significant increase in the mean diameter of yolky follicles in Group-III (25,000ppm zinc/Kg feed) dose ($P<0.05$) but Group-II (off fed) and Group-IV (30,000ppm zinc/Kg feed) did not show significant difference compared to controls. One way analysis of variance showed highly significant differences in means of different groups $F_{(3,116)}=3.19$; $P=0.03$

In categories from 5.1-10mm–25.1-30mm yolky follicles have no significant difference in mean follicular size in all treatment groups compared to control group. The results indicated that reversion to normal feed has improved the growth of yolky follicles in all the categories of follicles.

Mean Non Yolky Ovarian Follicular Diameter

Mean non yolky follicular diameter after the withdrawal of treatment period is given in Table 24. In this batch follicular diameter category range from ≤200µm to >1001µm did not show significant difference compared to Group-I in all the treatment groups. There was no significant difference in follicular diameter in comparisons among treatment groups as well. This indicates reversion of birds to normal feed has improved the follicular growth. The was a conspicuous change after withdrawal of off fed and high zinc treatment, the non yolky follicles in the category of 801-1000µm and >1001µm

Mean Non Yolky Oocyte Diameter

Mean non yolky oocyte diameter is given in. Table 25. In Batch-II treatments were withdrawn to see whether effect of treatment persists after withdrawal or not. in which Compared to Group-I (controls), highly significant reduction in mean oocyte diameter in category ≤200µm was seen in Group-II ($t_{(75)}$=3.79; P=0.0003) and Group-IV ($t_{(64)}$=2.59; P=0.01). Group-II and Group-IV showed highly significant reduction in mean oocyte diameter ($t_{(87)}$=4.01; P=0.0001; $t_{(76)}$=2.74; P=0.008) compared to Group-III in the same category (≤200µm). In oocyte diameter category 201-400µm in Group-II and Group-IV showed significant reduction in mean oocyte diameter ($t_{(74)}$=2.38; P=0.02; $t_{(85)}$=4.38; P<0.0001 respectively) compared to control. In same category (201-400µm) Group-II and Group-IV showed highly significant reduction in mean oocyte diameter ($t_{(60)}$=3.72; P=0.0001; $t_{(76)}$=2.74; P=0.008 respectively) compared to Group-III

Compared to control in oocyte diameter category 401-600µm of Group-II and Group-IV ($t_{(51)}$=3.87; P=0.0003; $t_{(75)}$=5.36; P<0.0001 respectively) highly significant decrease was observed. Compared to Group-III of same category there was significant decrease in oocyte diameter in Group-II and Group-IV ($t_{(60)}$=3.72; P=0.0004; $t_{(84)}$=5.28; P<0.0001 respectively). In category 601-800µm mean oocyte diameter in Group-II and Group-IV ($t_{(13)}$=3.15; P=0.008; $t_{(25)}$=2.66; P=0.01 respectively) showed highly significant decrease compared to that of Group-I. Mean oocyte diameter in category range 801-1000µm reduced significantly ($t_{(19)}$=3.37; P=0.003; $t_{(16)}$=2.67; P=0.01 respectively) in Group-II and Group-IV compared to Group-I. Group-II and

Group-IV also significant reduction ($t_{(14)}$=3.09; P=0.008; $t_{(11)}$=2.67; P=0.02 respectively) in mean oocyte diameter of same category 801-1000µm compared to Group-III. No difference was seen in mean oocyte diameter of category >1001µm among all groups. These results showed off fed condition and high zinc dose (30,000ppm zinc/Kg feed) has severely affected the oocyte diameter than with low .zinc dose treatment (25,000ppm zinc/Kg feed)

Mean Non Yolky Follicular Wall Thickness

Mean follicular wall thickness (µm) categories in the four treatment groups after withdrawal of treatment, is given in Table 26. Compared to control (Group-I) there was highly significant decrease in mean follicular wall thickness in Group-II ($t_{(75)}$=7.06; P<0.0001) in category ≤20µm and Group-II also showed highly significant decrease in follicular wall thickness in same category (≤20µm) compared to Group-III ($t_{(87)}$=4.29; P<0.0001) and Group-IV ($t_{(81)}$=4.02; P<0.0001). In category 21-40µm follicular wall thickness in Group-II compared to Group-I showed highly significant decrease ($t_{(47)}$=4.59; P<0.0001). Similarly, Group-II compared to Group-III and Group-IV in category 21-40µm follicular wall thickness decreased ($t_{(74)}$=6.68; P<0.0001; $t_{(81)}$=4.31; P<0.0001) highly significantly. In category 21-40µm Group-III showed significant increase ($t_{(85)}$=2.67; P=0.009) in follicular wall thickness compared to Group-IV. Follicular wall thickness in category 41-60µm in Group-II, Group-III and Group-IV reduced significantly ($t_{(51)}$=4.29; P<0.0001; $t_{(61)}$=2.33; P=0.02; $t_{(75)}$=3.22; P=0.002 respectively) compared to Group-I. Group-II also showed significant decrease ($t_{(60)}$=2.48; P=0.02) in follicular wall thickness compared to Group-III. In Group-II and Group-IV mean follicular wall thickness of category 61-80µm decreased ($t_{(14)}$=2.95; P=0.01; $t_{(75)}$=3.01; P=0.006) significantly compared to Group-I. In categories 81-100µm and >101µm, compared to control (Group-I), treatment groups, i.e. Group-II, Group-III and Group-IV did not show significant difference. In follicular wall severe effect on reduction in its thickness has been seen due to off fed condition as well as due to high dose of zinc(30,000ppm zinc/Kg feed).

Mean Non Yolky Ovarian Follicle Number

Mean number of follicle in control and all treatment groups after the withdrawal of treatment and resumption to normal feed in different categories are shown in Table 27. In this batch follicular number in category range from ≤200µm to >1001µm in all the treatment groups did not show significant difference compared to Group-I. There was also no significant difference in follicular number in comparisons among treatment groups as well. However, in categories 601-800µm, 801-1000µm and >1001µm follicles had developed in Group-II and Group-IV which were absent in Bath-I.

7 HISTOMORPHOLOGY

General and Histological Observations of Ovaries

In this batch treatment was withdrawn and birds were kept for fifteen (15) days on normal feeding before slaughtering. Ovarian Weight and size in off fed, low and high zinc dose treatment groups increased during this period and was comparable to Group-I (Table 17). After slaughtering the birds their ovaries were removed carefully and large yolky and small whitish (transparent) yolky follicles were counted and measured (Table 22 and 23). In ovary of control and treated birds yellow yolky follicles were observed whereas whitish small yolky follicles were less in treated birds. Blood vessels were visible in yolky follicles. Microscopic study of cross section of middle potion of ovary of the White Leghorn layer birds at 25th week of age ovary showed that it possessed two distinct regions, an outer cortex and inner medulla. The cortex contained follicles of variable size range. In between the follicles abundant compactly arranged stromal tissue was present. The inner medullar stroma was composed of well vascularised and innervated connective tissue with spaces (lacunae), nerves and blood vessels.

The Control ovary contained number of healthy small and large follicles of diameter category ranging from ≤200 to >1001µm at various developmental stages were seen. Number of ovarian follicles in Group-II, Group-III and Group-IV of these categories also increased compared to control group (Fig. 22A). Follicles of larger categories were present both in zinc treated groups (Fig. 22CD) and off fed group (Fig. 22B) after the withdrawal of treatment (Table 24 and 25).

The internal structure of oocyte of follicles had improved and possessed well defined spherical nucleus with distinct network of chromatin and clear nuclear membrane in control (Fig. 23A) and all treatment groups (Fig. 23BCD). Normal feeding of the birds improved the structure of nucleus and nuclear matrix.

There was no difference in structure of primordial follicles in treated groups compared to control (Fig. 24A). In this batch treated bird ovaries (Fig. 24BCD) possessed normal ooplasm in the follicles. In these follicles oocyte was attached to the granulosa layer with clear cytoplasmic extension.

The thin layer of zona pellucida between ooplasm and granulosa layer of follicle, with well defined cytoplasmic processes was seen in control (Fig. 25A) and all treated groups (Fig. 25BCD) after the withdrawal of treatment and resumption to the normal feed.

Large follicles oocyte was surrounded by a single layer of granulosa cells with strong basal membrane. Granulosa layer and basal lamina were normal in control (Fig. 26A) and all groups (Fig. 26BCD) after the withdrawal of treatment. Although the thickness of peripheral follicular epithelial (theca interna and theca externa) and granulosa layers was increased but it was still lesser in all categories of follicles in off fed and low and high dose zinc treated groups compared to control.

In this batch the thecal layer was distinguishable into two portions, theca interna and theca externa. Follicular epithelium layer (theca interna and theca externa) in follicles of control group (Fig. 27A) and all treated birds was compactly arranged with clear thecal glands (Fig. 27BCD).

Theca interna contained thecal glands in control and all treatment groups However, the concentration of thecal gland in control group (Fig. 28A) was higher compared to all treatment groups (Fig. 28BCD).

The normal feeding also improved the health of ovarian follicles. Few atretic primordial follicles were observed in treated groups (Fig. 29ABCD).

Loosely arranged stromal tissue in treated groups improved and there were less spaces in interstitial tissue due to normal feeding of treatment groups. Compact and well

organized stromal tissue was seen in control (Fig. 30A) and all groups with smooth connective tissue (Fig. 30BCD).

Table 17: Mean ovarian, oviduct and liver weight (gm), length and width (mm) in young White Leghorn(WLH) layer birds slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

Treatment groups	Ovaries			Oviduct		Liver
	Weight	Length	width	weight	Length	Weight
Group-I	48.11±4.15	36.35±3.07	17.36±1.08	54.69±1.28	66.61±0.67	60.24±1.89
Group-II	51.16±2.45	37.67±2.29	17.60±0.73	46.61±1.18^{a***}	59.11±2.52^{a**}	34.27±1.66^{a****}
Group-III	48.83±2.22	35.68±2.87	18.49±1.41	48.53±1.31^{a***}	63.19±1.99	40.33±2.46^{a****}
Group-IV	48.46±2.23	37.92±2.51	17.57±3.22	45.25±1.26^{a****}	62.05±1.72^{a*}	39.34±1.67^{a****}

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 19: Mean plasma estradiol concentration (pg/ml) in White Leghorn (WLH) layer birds slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Group I	**Group-II**	**Group-III**	**Group-IV**
3rd day	204.11±13.38	77.15±6.64^{a****}	86.57±8.55^{a***}	73.41±6.46^{a***}
6th day	222.99±15.17	121.58±8.54^{a****}	115.19±9.09^{a****}	118.67±15.48^{a****}
9th day	215.99±8.69	137.14±7.23^{a****}	159.23±14.61^{a***}	149.51±16.87^{a***}
12th day	225.23±10.15	147.12±17.19^{a***}	170.36±12.78^{a***}	172.37±17.88^{a*}
15th day	229.10±10.74	165.61±13.20^{a***}	176.49±9.42^{a***}	182.46±9.59^{a***}

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 20: Changes in mean plasma progesterone concentration (ηg/ml) in young White Leghorn (WLH) Layer birds slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Group I	Group-II	Group-III	Group-IV
3rd day	4.19±0.25	2.13±0.27^{a****}	2.60±0.06^{a****}	2.38±0.04^{a****}
6th day	4.39±0.19	3.21±0.21^{a***}	3.10±0.05^{a****}	3.22±0.06^{a****}
9th day	4.66±0.12	3.67±0.29^{a***}	3.61±0.02^{a****}	3.89±0.20^{a***}
12th day	4.29±0.05	3.78±0.17^{a**}	3.56±0.32^{a*}	3.99±0.13
15th day	4.50±1.01	3.88±0.15	4.00±0.06	4.07±0.19

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 21: Changes in mean plasma corticosterone concentration (ηg/ml) in young White Leghorn (WLH) layer birds slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Group I	Group-II	Group-III	Group-IV
3rd day	8.30±1.73	37.28±0.53^{a****}	34.71±3.07^{a****}	35.39±0.45^{a****}
6th day	8.38±2.12	29.13±2.92^{a****}	27.49±1.74^{a****}	29.76±0.80^{a****}
9th day	8.91±1.64	24.58±3.62^{a***}	23.41±2.05^{a****}	21.04±1.10^{a****}
12th day	8.39±2.77	19.49±3.95^{a*}	21.28±0.59^{a****}	22.63±0.60^{a****}
15th day	8.92±2.09	16.95±4.28^{a**}	17.57±0.85^{a***}	18.48±0.88^{a***}

Values are means±SEM (N=5)
a= Group-I compared to all other groups.
b= Group-II compared to Group-III and group-IV.
c= Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 22: Mean ovarian yolky follicles number of different category range (mm) in White Leghorn layer birds at 25th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Yolky Follicles Number Category Range (mm)					
	1-5mm	**5.1-10mm**	**10.1 -15mm**	**15.1-20mm**	**20.1-25mm**	**25.1-30mm**
Group I	115.20±4.32	1.80±0.58	0.80±0.37	1.80±0.73	1.00±0.75	1.60±0.51
Group II	61.40±4.57[a***]	0.60±0.24	(2)	1.20±0.20	1.40±0.75	(1)
Group III	70.20±5.27[a***]	1.00±0.45	0.80±0.37	1.00±0.55	1.60±0.93	1.40±0.51
Group IV	60.40±2.69[a***]	1.00±0.45	0.80±0.37	(2)	1.00±0.55	1.40±0.51

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001
Digits in parenthesis are number of yolky follicle seen in respective group

Table 23: Mean yolky follicles diameter (mm) in different category range in White Leghorn layer birds at 25th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Follicular Category Range (mm)					
	1-5mm	**5.1-10mm**	**10.1 -15mm**	**15.1-20mm**	**20.1-25mm**	**25.1-30mm**
Group I	3.73±0.59	6.20±0.55	14.34±0.22	17.65±0.47	22.44±0.55	27.72±0.33
Group II	4.00±0.30	6.17±0.34	12.04 (2)	16.67±0.58	23.57±0.36	27.97 (1)
Group III	4.47±0.28[a*]	6.33±0.60	14.09±0.36	17.37±0.85	22.90±0.79	26.98±0.49
Group IV	3.98±0.29	6.95±0.45	12.50±0.37	18.89 (2)	22.48±0.82	26.18±0.54

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001
Digits in parenthesis are number of yolky follicle seen in respective group

Table 24: Mean non yolky follicular diameter (µm) in White Leghorn layer birds at 25th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Mean Follicular Diameter Categories (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group I	144.97±7.78	338.65±17.26	525.22±13.00	725.64±26.04	904.19±15.53	1210.25±61.43
Group II	132.39±6.40	308.08±9.51	505.90±10.92	709.12±25.62	892.28±21.95	1140.31±24.58
Group III	148.36±6.76	319.33±9.95	507.44±9.20	703.25±21.09	914.49±19.15	1128.13±75.07
Group IV	141.22±5.89	317.63±8.93	513.02±8.33	721.35±11.69	934.43±29.24	1165.40±49.45

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 25: Mean non yolky oocyte diameter (µm) in White Leghorn layer birds at 25th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Mean Oocytes Diameter Categories (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group I	123.65±7.64	312.11±17.29	494.40±13.52	689.10±29.22	868.88±14.76	1148.91±58.65
Group II	94.16±3.87^{a****}	260.66±8.51^{a***}	430.64±9.18^{a****}	595.08±15.48^{a***}	784.16±21.34^{a***}	1018.59±8.41
Group III	124.37±6.68^{b****}	292.18±9.93^{b**}	480.02±9.14^{b****}	618.04±27.88	865.10±15.13^{b***}	1065.63±62.79
Group IV	101.64±4.44^{ac***}	238.98±7.34^{ac****}	416.05±7.89^{ac****}	614.65±11.63^{a***}	794.36±23.44^{a***c**}	981.69±51.76

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 26: Mean non yolky follicular wall thickness (µm) in White Leghorn layer birds at 25th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Mean Follicular Wall Thickness Categories (µm)					
	≤20µm	**21-40µm**	**41-60µm**	**61-80µm**	**81-100µm**	**>101µm**
Group I	16.61±0.50	30.22±1.75	55.68±1.23	75.62±1.18	91.26±1.69	123.07±6.23
Group II	12.31±0.37^{a****}	23.54±0.61^{a****}	48.80±1.02^{a****}	68.23±1.91^{a***}	88.48±0.21	106.58±5.07
Group III	15.44±0.65^{b****}	30.45±0.81^{b****}	52.20±0.91^{ab**}	71.86±2.31	87.58±1.17	121.28±5.83
Group IV	15.86±0.47^{b****}	27.64±0.69^{b****c***}	50.39±1.01^{a***}	71.08±0.73^{a***}	89.16±2.76	115.19±1.62

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 27: Mean number of non yolky follicles per section of ovary in different category range in White Leghorn layer birds at 25th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Mean Follicle Number Category Range (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group-I	31.80±4.87	10.80±1.36	7.80±0.86	3.80±0.66	2.60±0.87	1.80±0.37
Group-II	27.40±4.87	8.20±1.39	6.20±1.56	3.00±0.71	2.20±1.02	1.40±0.40
Group-III	26.20±3.93	9.80±0.66	8.40±2.09	3.60±0.51	2.40±0.60	2.00±0.55
Group-IV	28.80±1.66	8.80±1.07	5.40±1.21	2.40±0.68	1.60±0.40	1.60±0.40

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

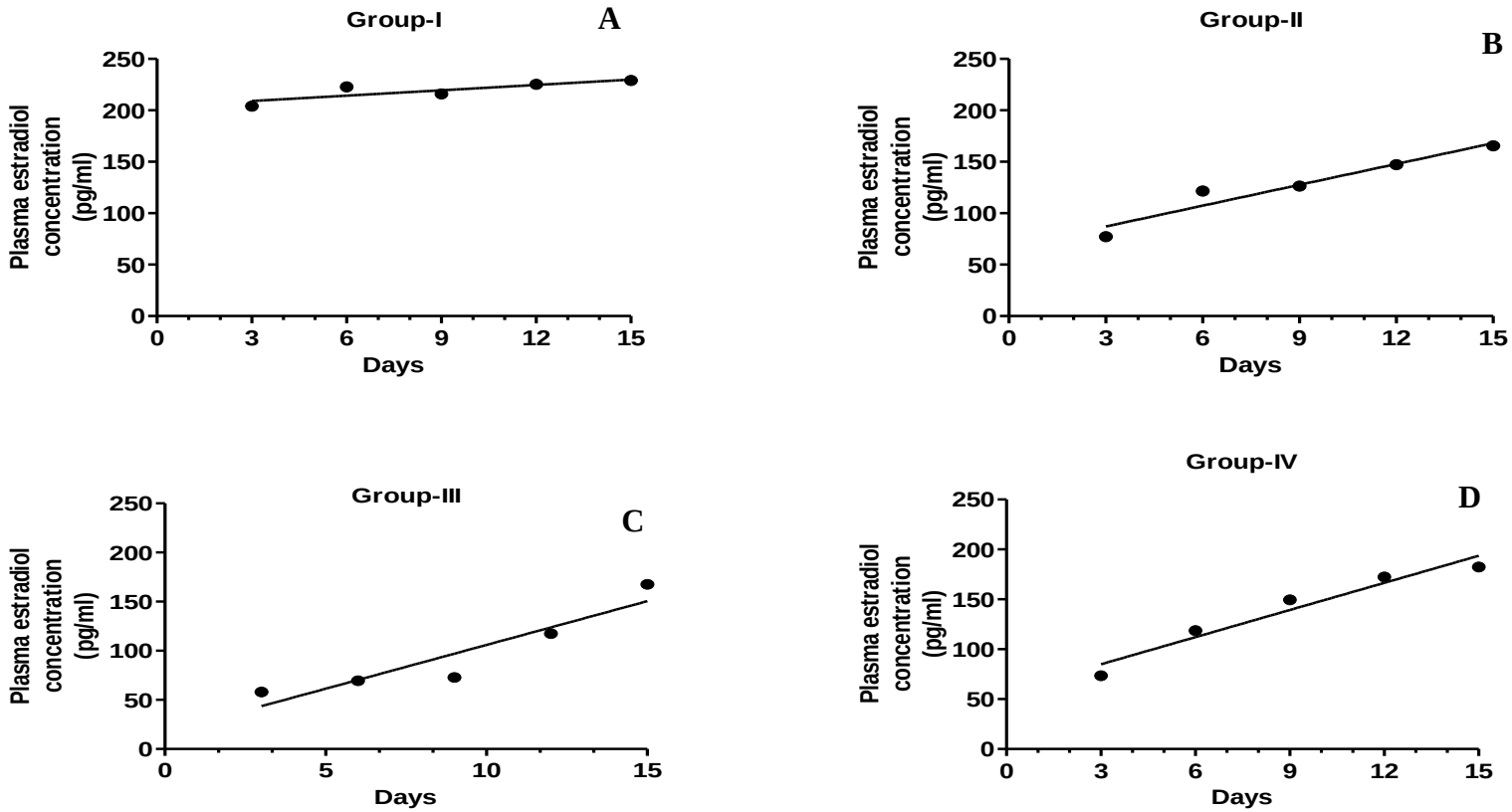


Fig. 19: Regression line showing non significant increase in mean plasma estradiol levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during fifteen days after withdrawal of treatment in White Leghorn layer birds at 25th week of age (Batch-II).

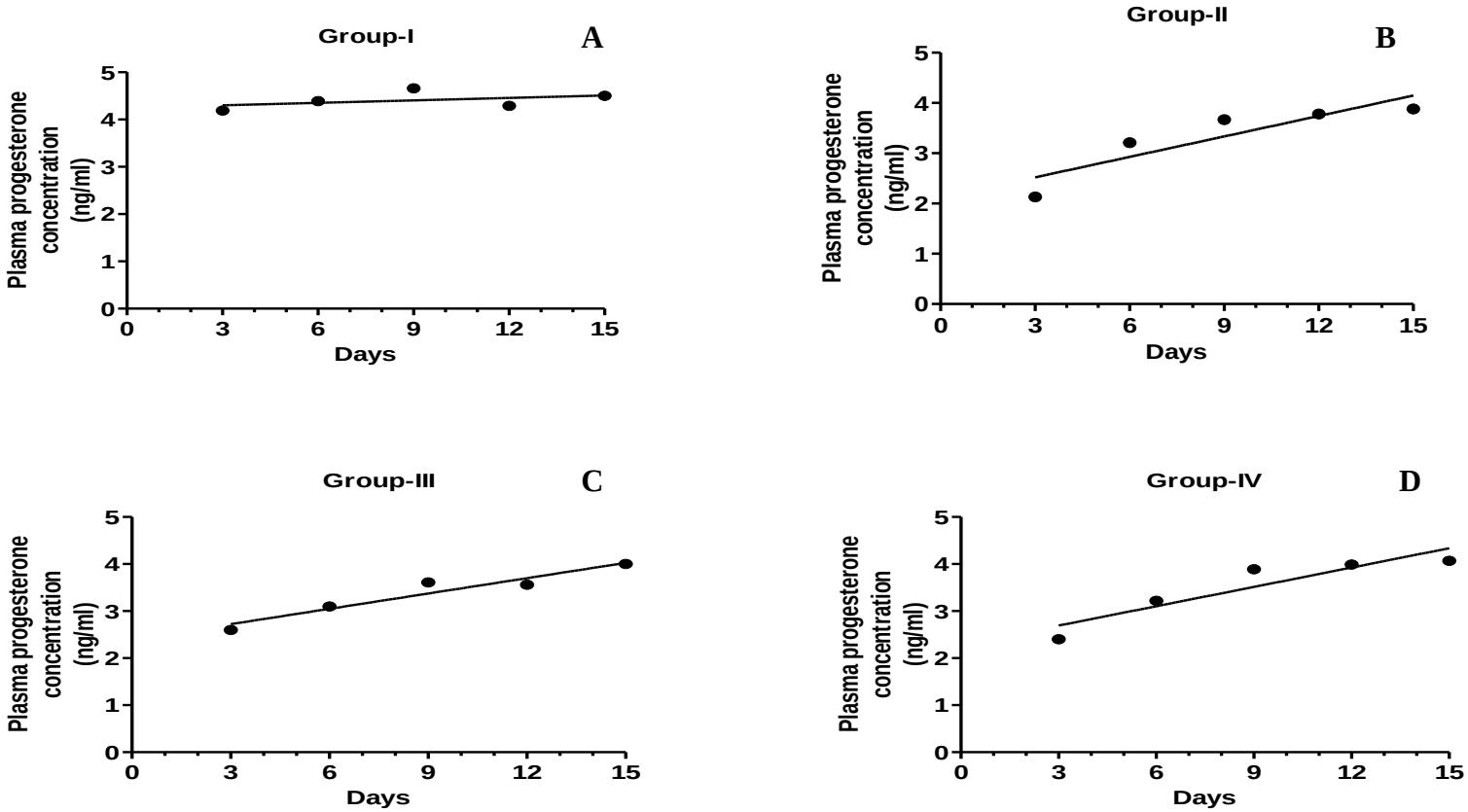


Fig. 20: Regression line showing non significant increase in mean plasma Progesterone levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during fifteen days after withdrawal of treatment in White Leghorn layer birds at 25th week of age (Batch-II).

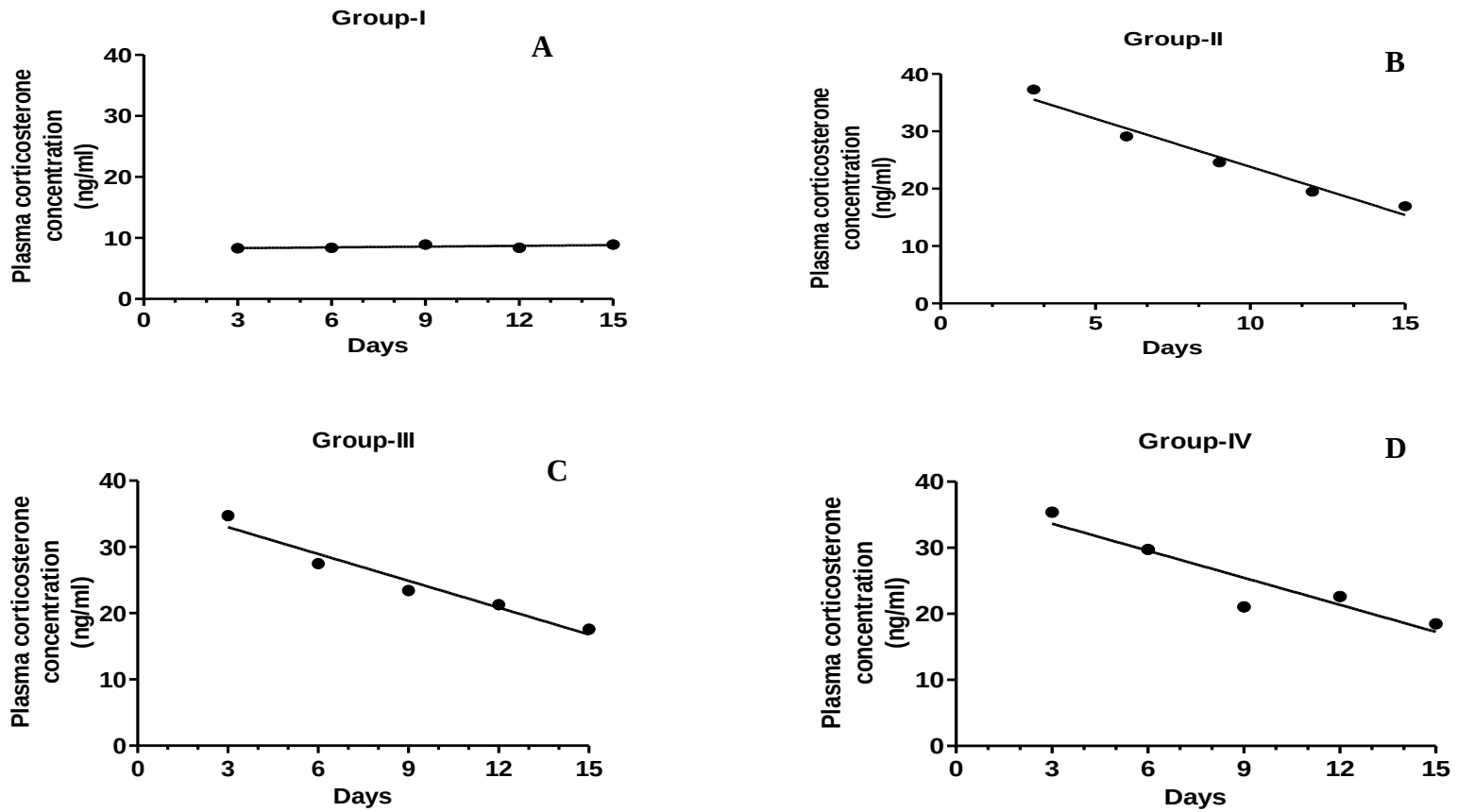


Fig. 21: Regression line showing non significant decrease in mean plasma corticosterone levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during fifteen days after withdrawal of treatment in White Leghorn layer birds at 25th week of age (Batch-II).

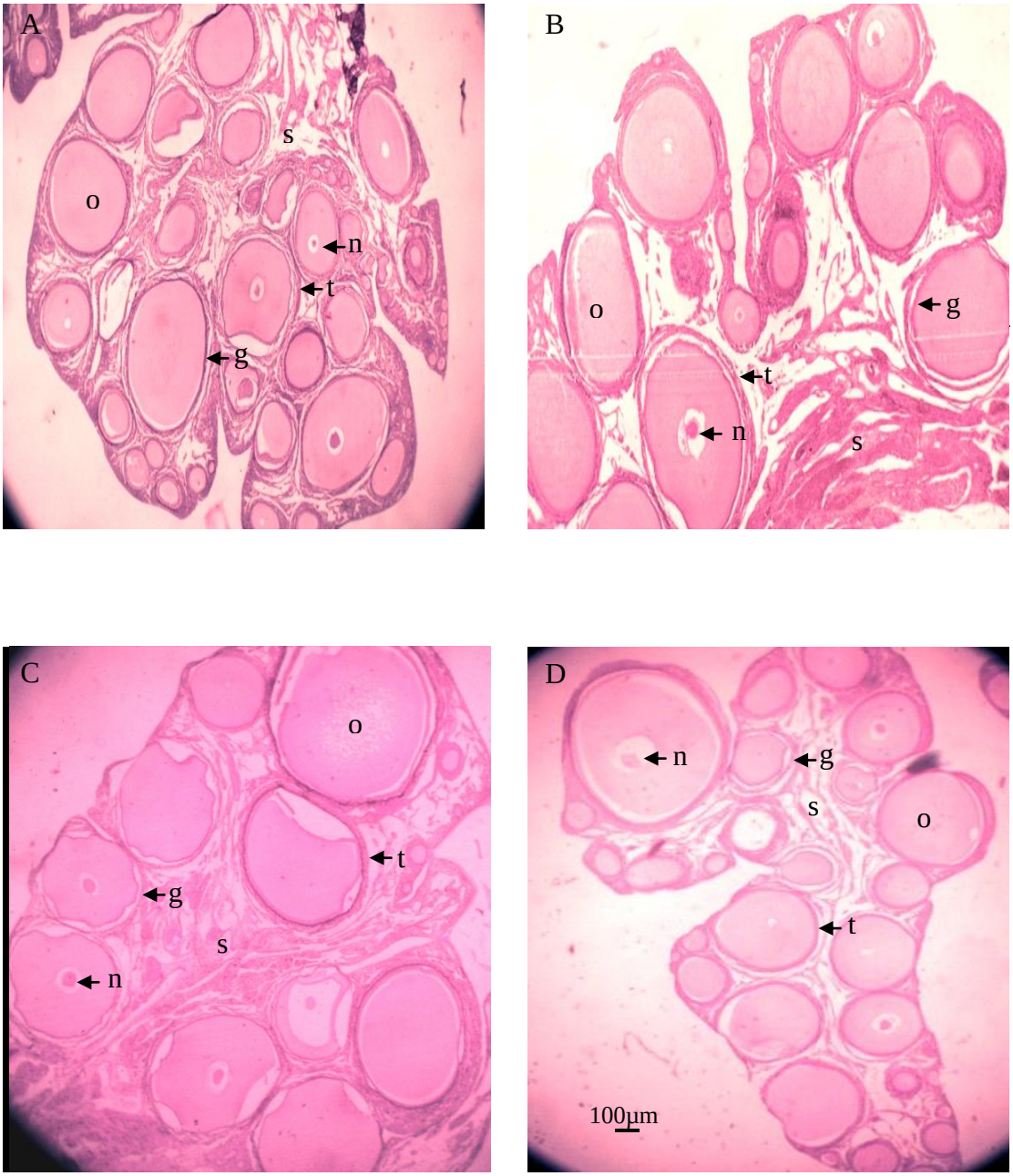


Fig 8: Photomicrograph of the middle portion of the cross section of ovary showing follicles in control and treatment groups, birds slaughtered on 12th day of treatment at 25th week of age (Batch-I). (A) Control showing greater number of large and small follicles (o) oocyte, (t) thecal layer, (g) granulosa layer, (s) stromal tissue (n) nucleus (B) Group-II (off fed) and (C) zinc treated groups and (D) (Group-III and Group-IV) showing decrease in number of follicles (o) oocyte, (t) thecal layer, (g) granulosa layer, (s) stromal tissue (n) nucleus. Greater decrease in Group-II. H & E.

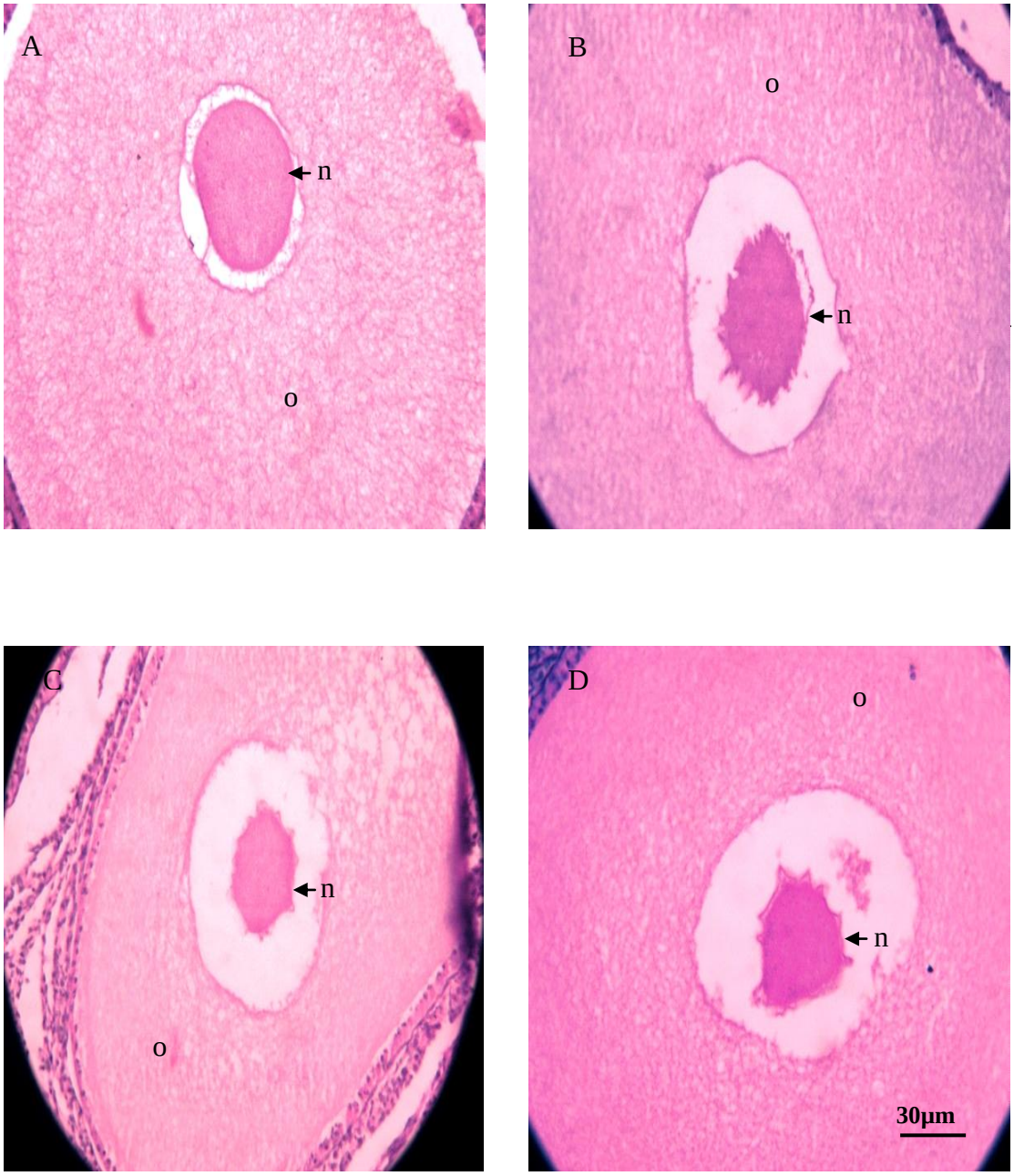


Fig 9: Photomicrograph of the middle portion of the cross section of ovary showing oocyte nucleus in control and treatment groups, birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control showing normal spherical nucleus with clear nuclear membrane (o) oocyte, (n) nucleus (B) Group-II (off fed) (C) Group-III and (D) Group-IV (zinc treated groups) showing abnormal and deshaped nucleus with unclear nuclear membrane, (o) oocyte and (n) nucleus. H & E.

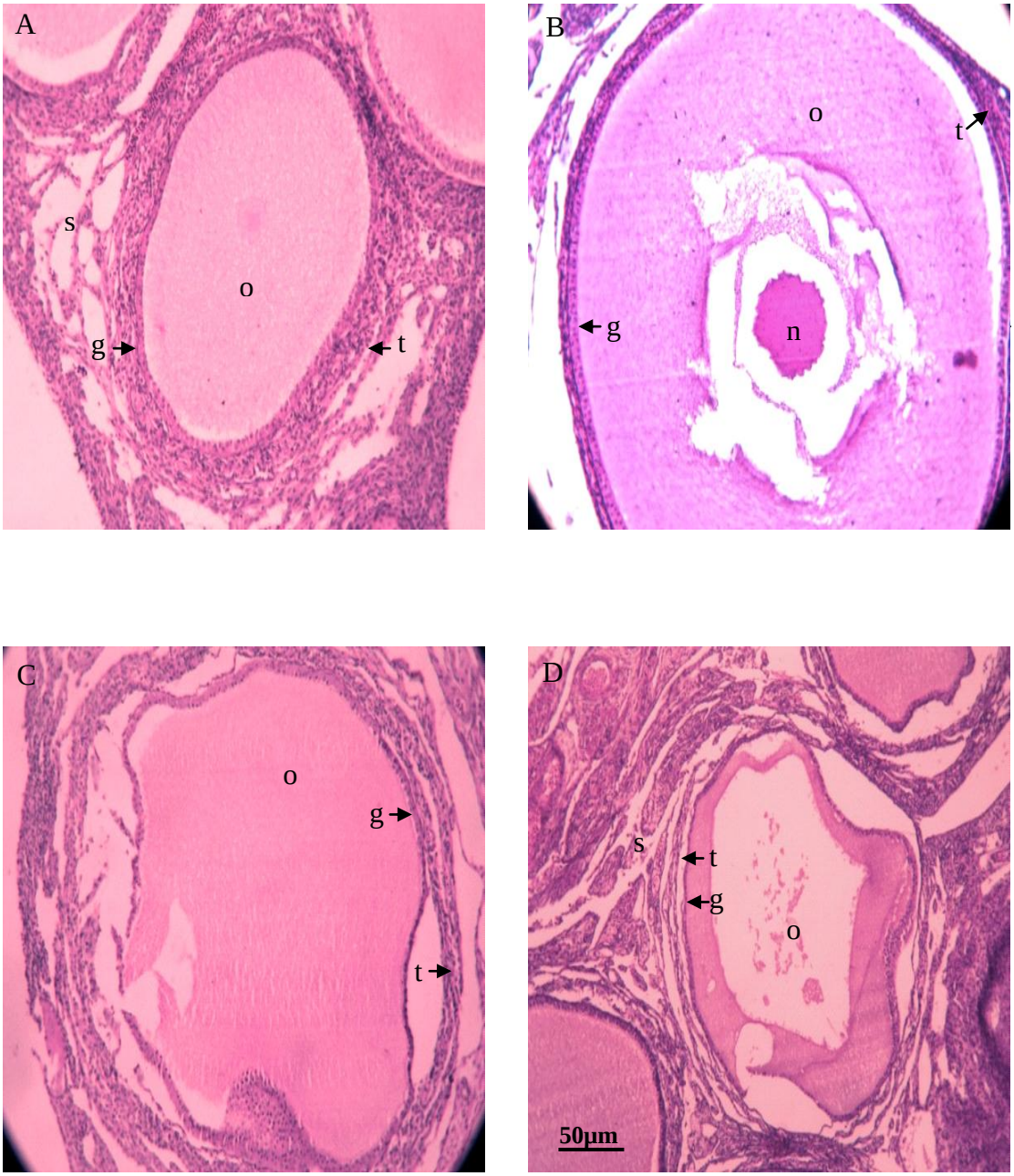


Fig 10: Photomicrograph of the middle portion of the cross section of ovary showing cytoplasm (ooplasm) of oocyte in control and treatment groups, birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control showing normal cytoplasm and intact thecal and granulosa layers (o) oocyte, (t) thecal layer, (g) granulosa layer, (B) Group-II (off fed) (C) Group-III and (D) Group-IV (zinc treated groups) showing abnormal cytoplasm, more disintegration in Group-II and Group-IV deshaped oocyte (o) oocyte, (t) thecal layer, (g) granulosa layer and (s) stromal tissue. H & E.

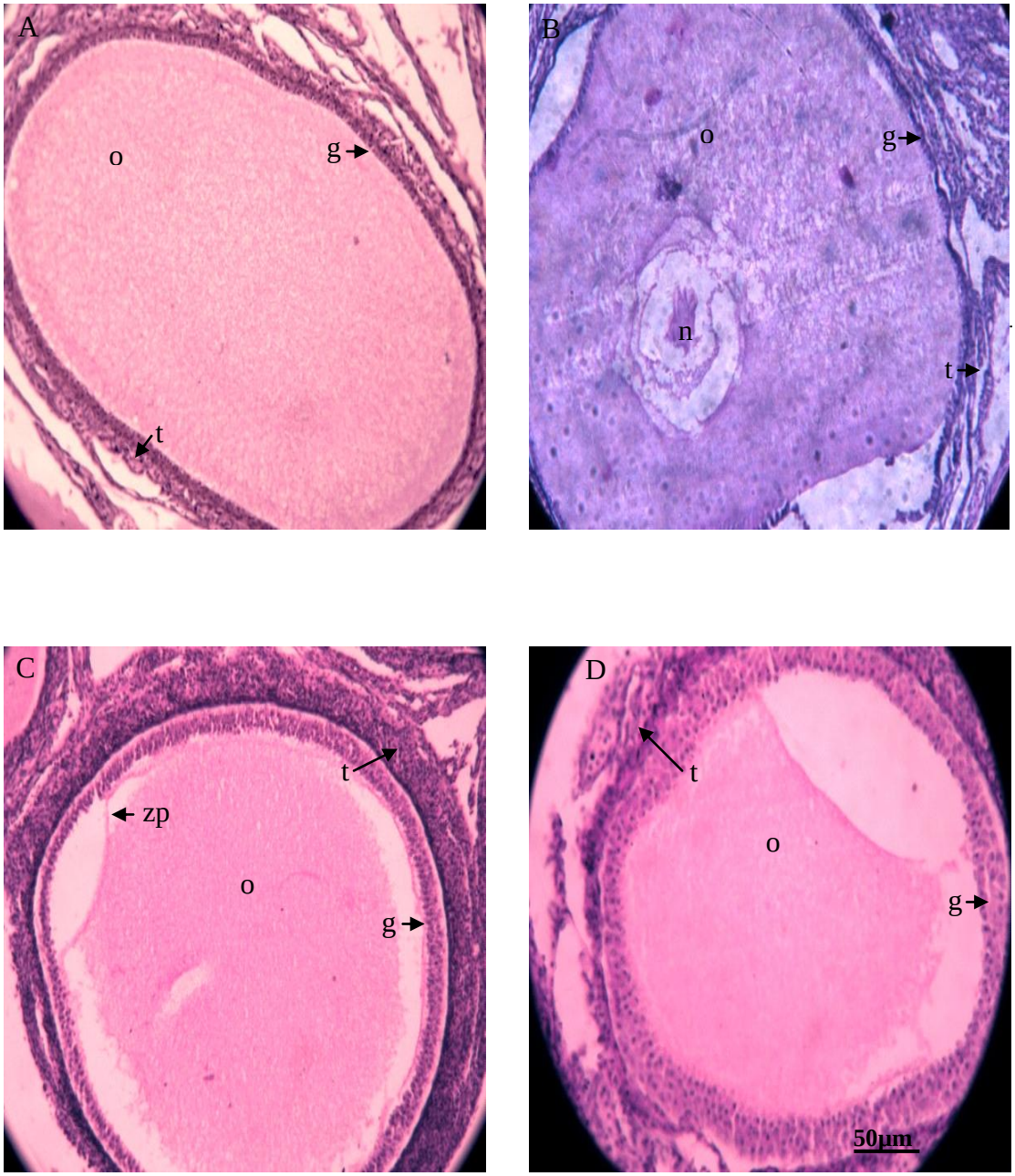


Fig 11: Photomicrograph of the middle portion of the cross section of ovary showing changes in zona pellucida in control and treatment groups, birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control showing normal zona pellucida attached with granulosa layer (o) oocyte, (t) thecal layer, (g) granulosa layer, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing disrupted zona pellucida layer (zp) zona pellucida, (o) oocyte, (t) thecal layer and (g) granulosa layer. H & E.

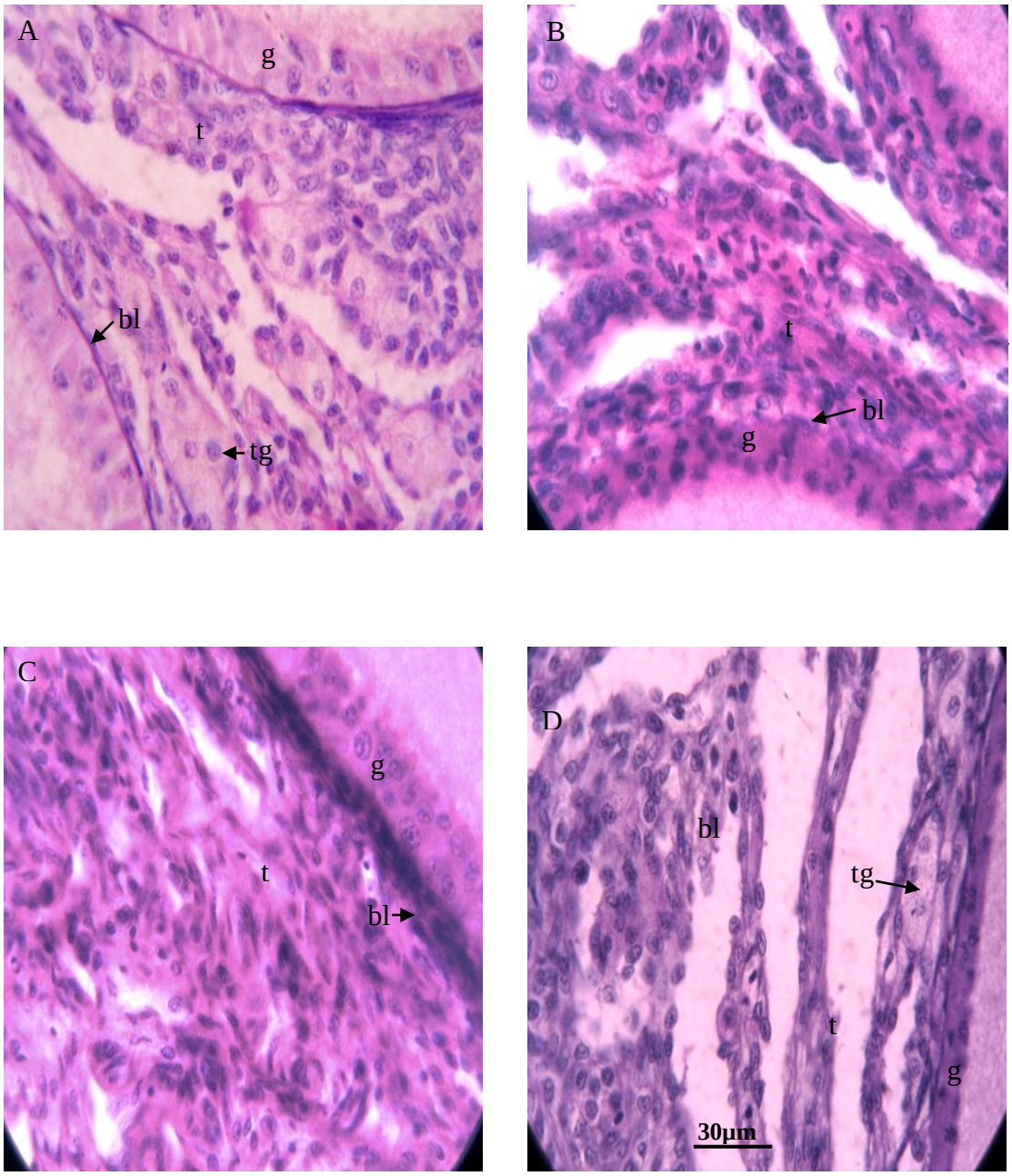


Fig 12: Photomicrograph of the middle portion of the cross section of ovary showing granulosa and basal lamina in control and treatment groups, birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control clear granulosa with basal lamina and thecal layer, (t) thecal layer, (g) granulosa, (bl) basal lamina, (tg) thecal gland (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing rough and intermingled basal and granulosa, (t) thecal layer, (g) granulosa, (bl) basal lamina and (tg) thecal gland. H & E.

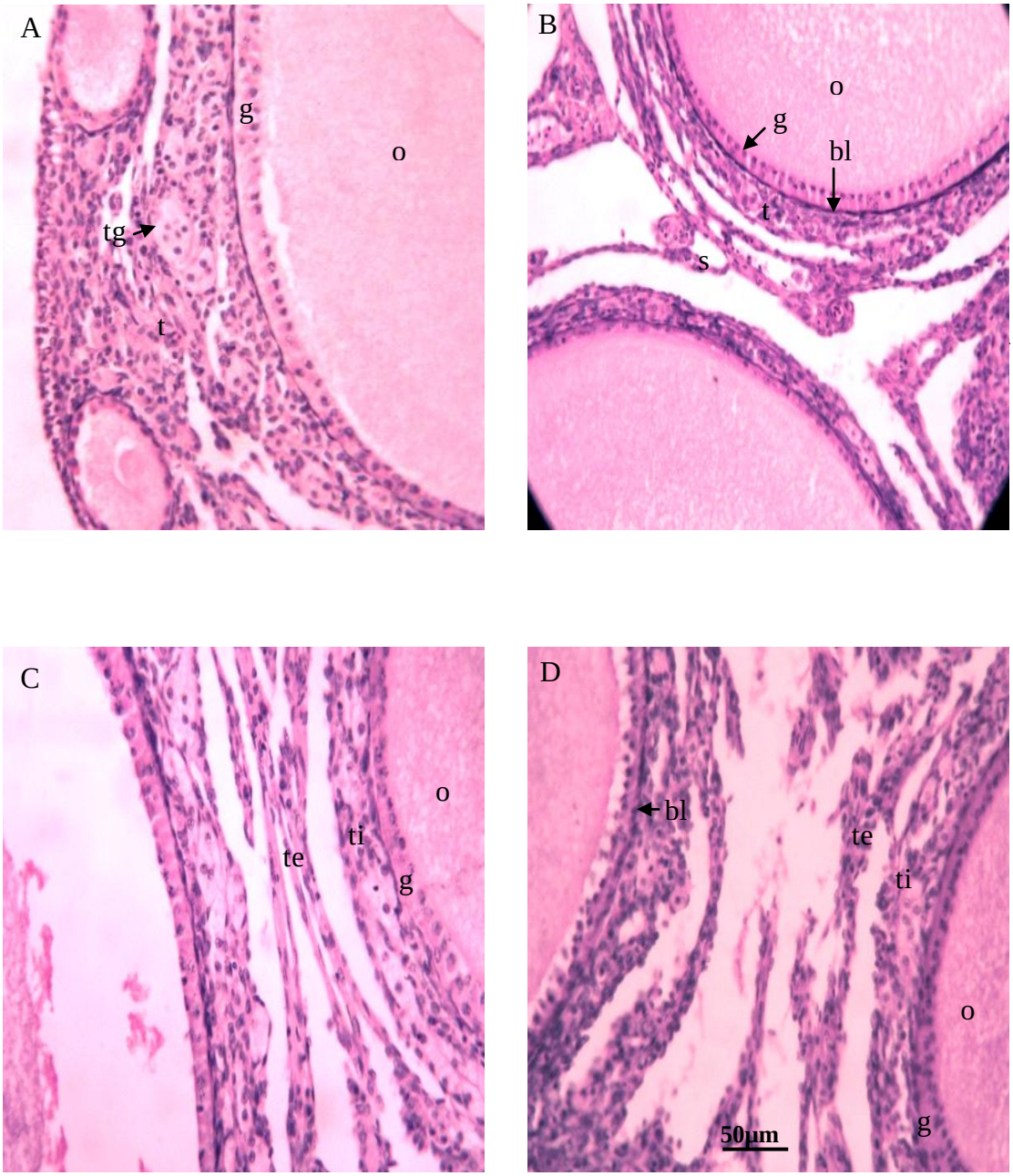


Fig 13: Photomicrograph of the middle portion of the cross section of ovary showing thickness of follicular wall in control and treatment groups birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control showing greater thickness of follicular layer, (tg) thecal gland, (g) granulosa, (o) oocyte, (t) thecal layer, (B) Group-II (off fed), (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing reduction in thickness of follicular wall, more in Group-II and Group-IV (ti) theca interna, (te) theca externa (g) granulosa, (o) oocyte and (bl) basal lamina. H & E

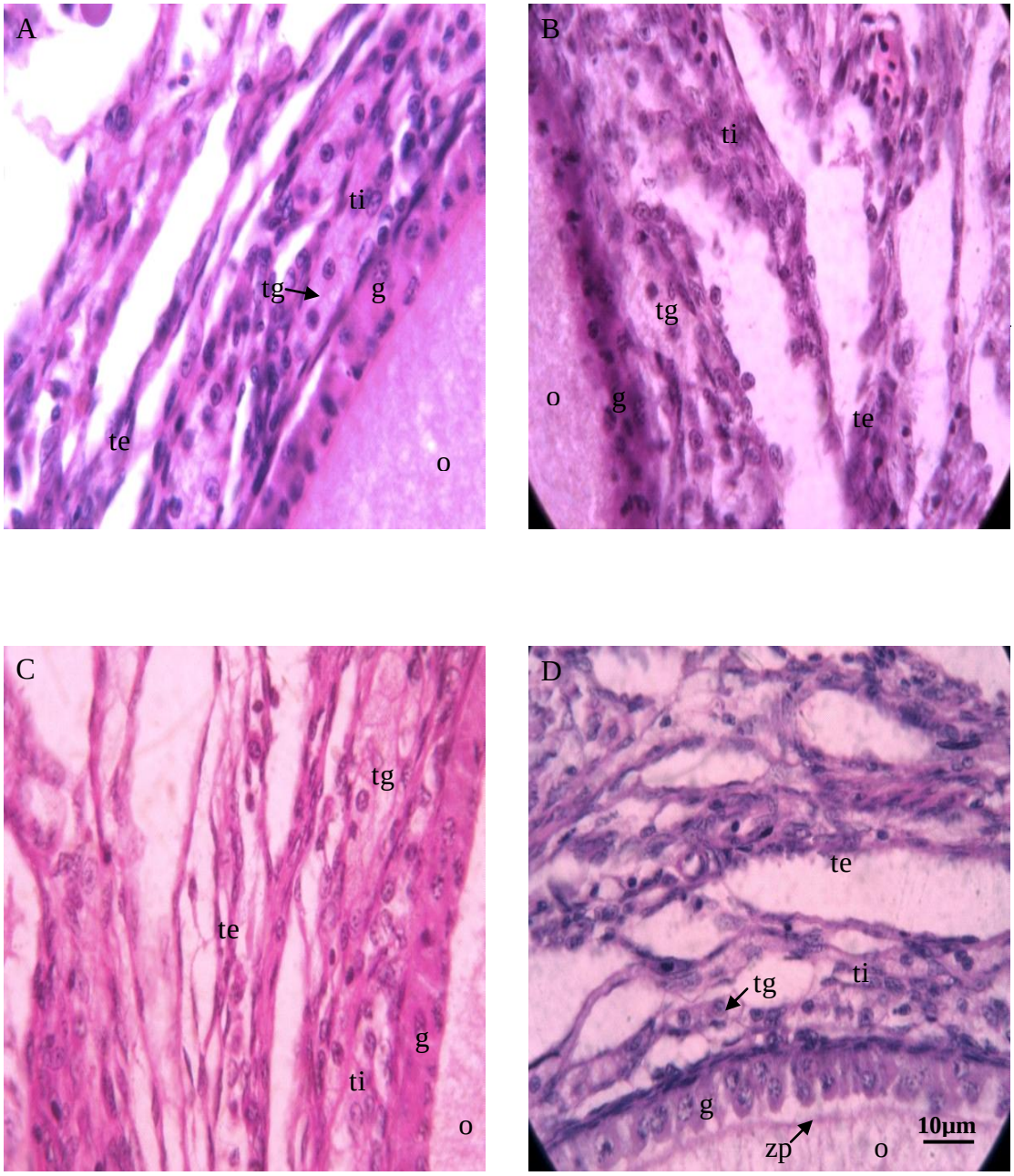


Fig 14: Photomicrograph of the middle portion of the cross section of ovary showing thecal gland concentration in control and treatment groups birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control showing greater number of thecal glands in thecal layer, (tg) thecal gland, (g) granulosa, (o) oocyte, (ti) theca interna (te) theca externa, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing less number of thecal gland in thecal layer (tg) thecal gland, (g) granulosa, (o) oocyte, (ti) theca interna (te) theca externa and (zp) zona pellucida. H & E.

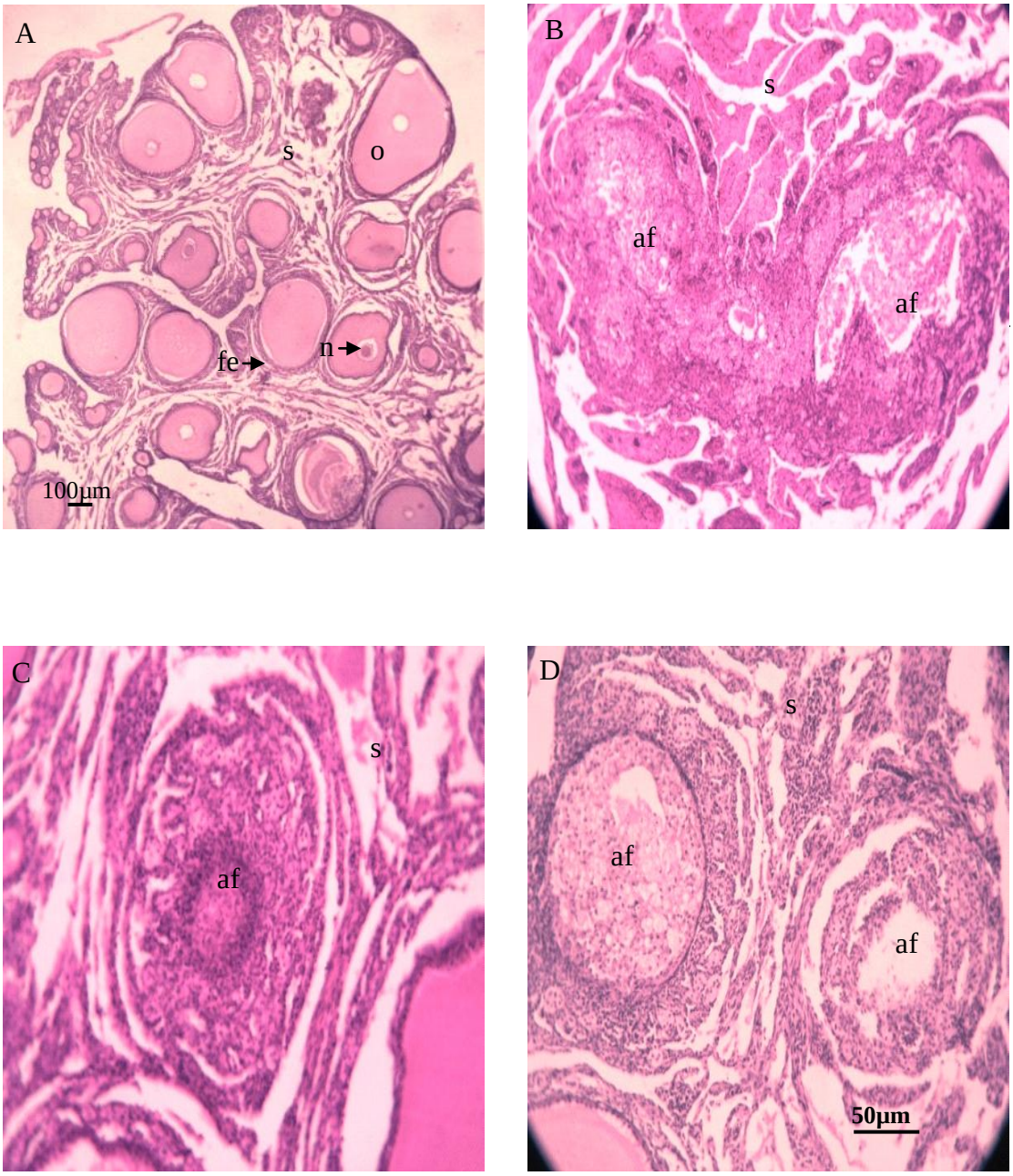


Fig 15: Photomicrograph of the middle portion of the cross section of ovary showing atretic follicle in control and treatment groups birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control showing no atretic follicle in follicle hierarchy, (fe) follicular epithelium (s) stroma, (o) oocyte, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing presence of atretic follicles, (af) atretic follicle and (s) stroma. H & E.

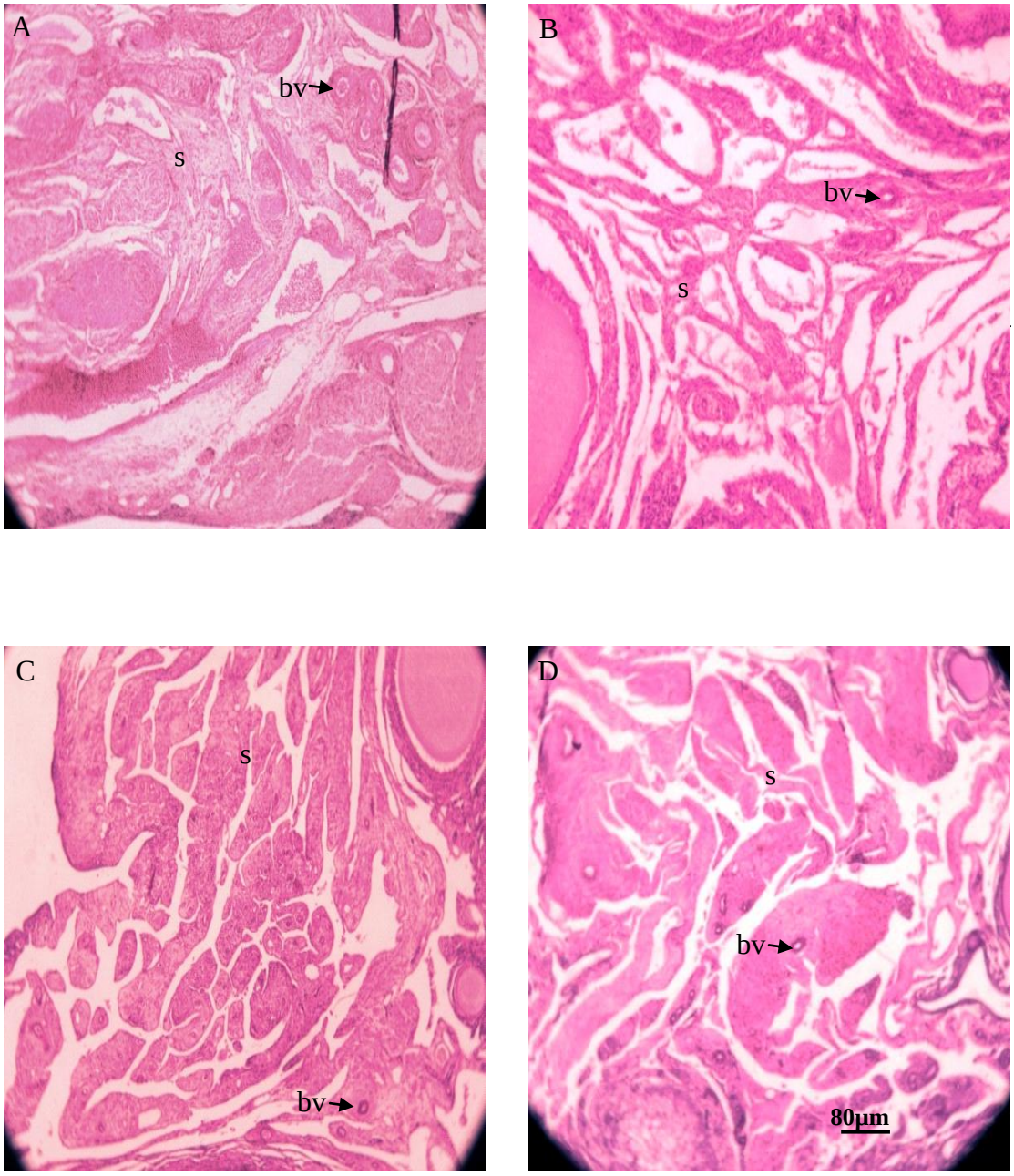


Fig 16: Photomicrograph of the middle portion of the cross section of ovary showing stromal tissue organization in control and treatment groups birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control showing compact organization of stromal tissue with numerous blood vessels (bv) blood vessels, (s) stroma, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing loose arrangement of stromal tissue (bv) blood vessel and (s) stroma. H & E.

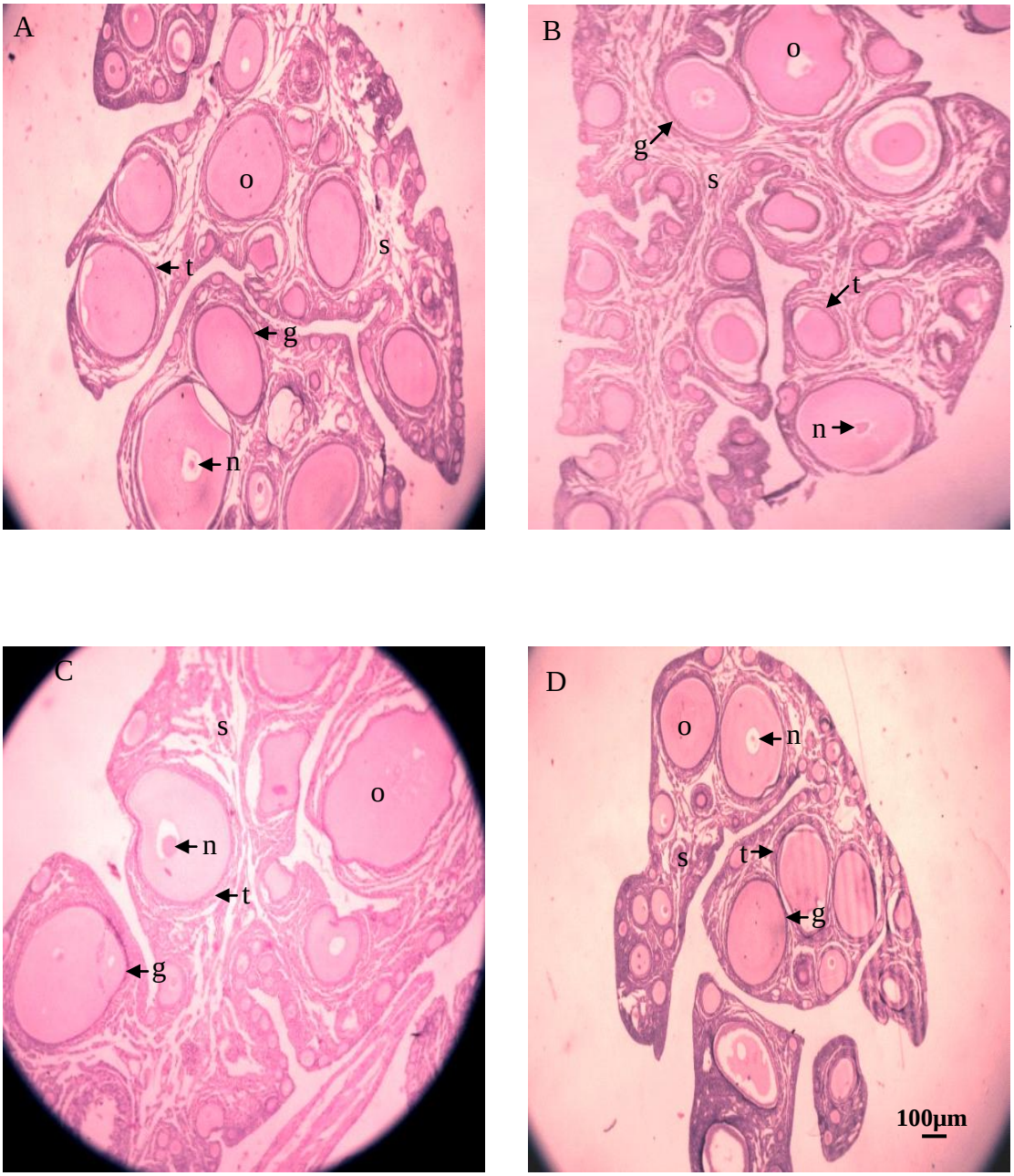


Fig 22: Photomicrograph of the middle portion of the cross section of ovary showing follicle in control and treatment groups, slaughtered on 15th day after withdrawal of treatment and resumption to normal feed at 25th week of age (Batch-II). (A) Control showing large and small follicles of all diameter categories (o) oocyte, (t) thecal layer, (g) granulosa layer, (s) stromal tissue, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing large and small follicles of all diameter categories (o) oocyte, (t) thecal layer, (g) granulosa layer, (s) stromal tissue and (n) nucleus. H & E.

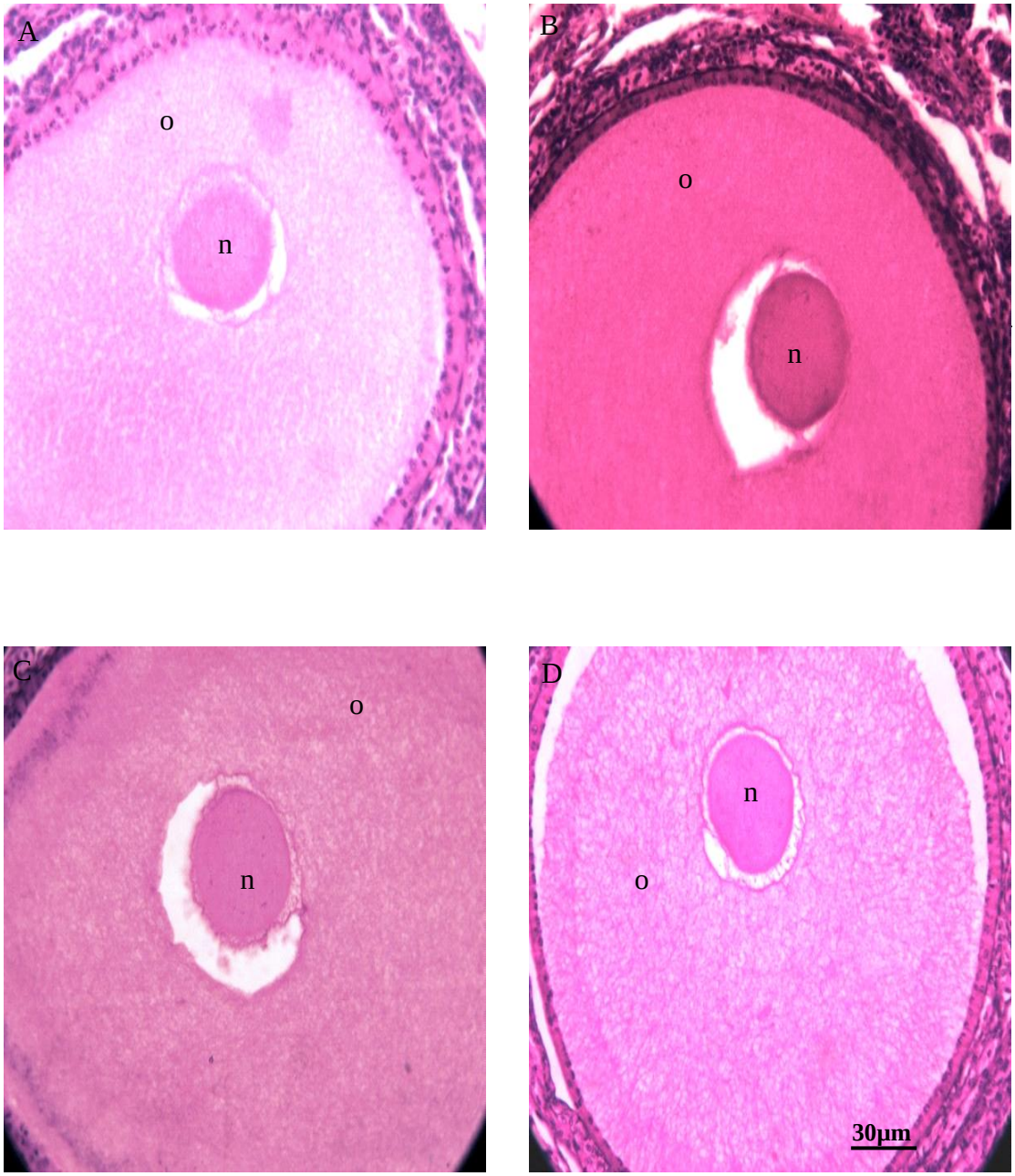


Fig 23: Photomicrograph of the middle portion of the cross section of ovary showing nucleus of follicle in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal feed at 25th week of age (Batch-II). (A) Control showing normal spherical nucleus with clear nuclear membrane (o) oocyte, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing normal spherical nucleus with clear nuclear membrane (o) oocyte and (n) nucleus. H & E.

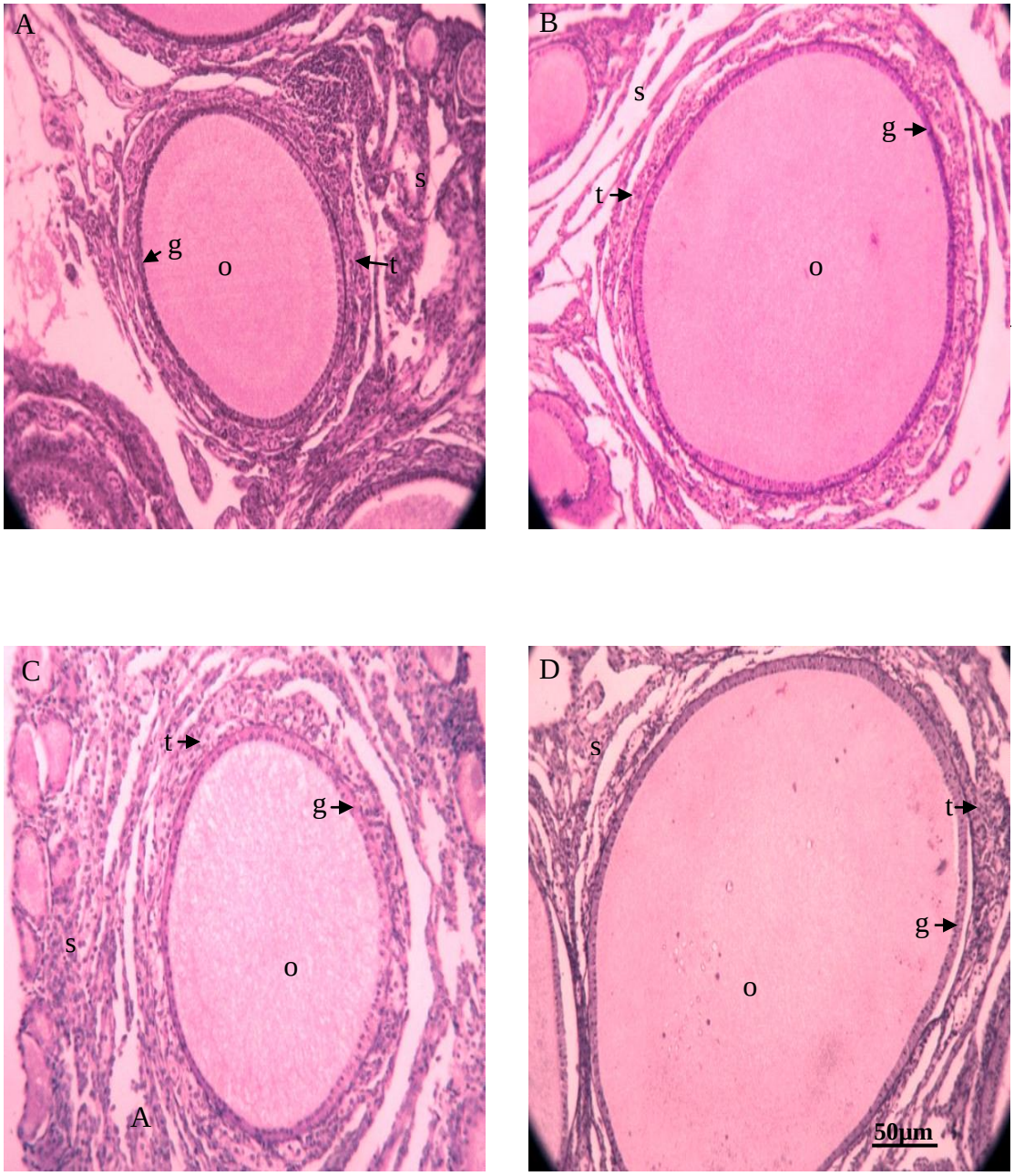


Fig 24: Photomicrograph of the middle portion of the cross section of ovary showing cytoplasm of follicle in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal feed at 25th week of age (Batch-II). (A) Control showing normal cytoplasm and intact thecal and granulosa layers (o) oocyte, (t) thecal layer, (g) granulosa layer, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing normal cytoplasm and intact thecal and granulosa layers (o) oocyte, (t) thecal layer and (g) granulosa layer. H & E.

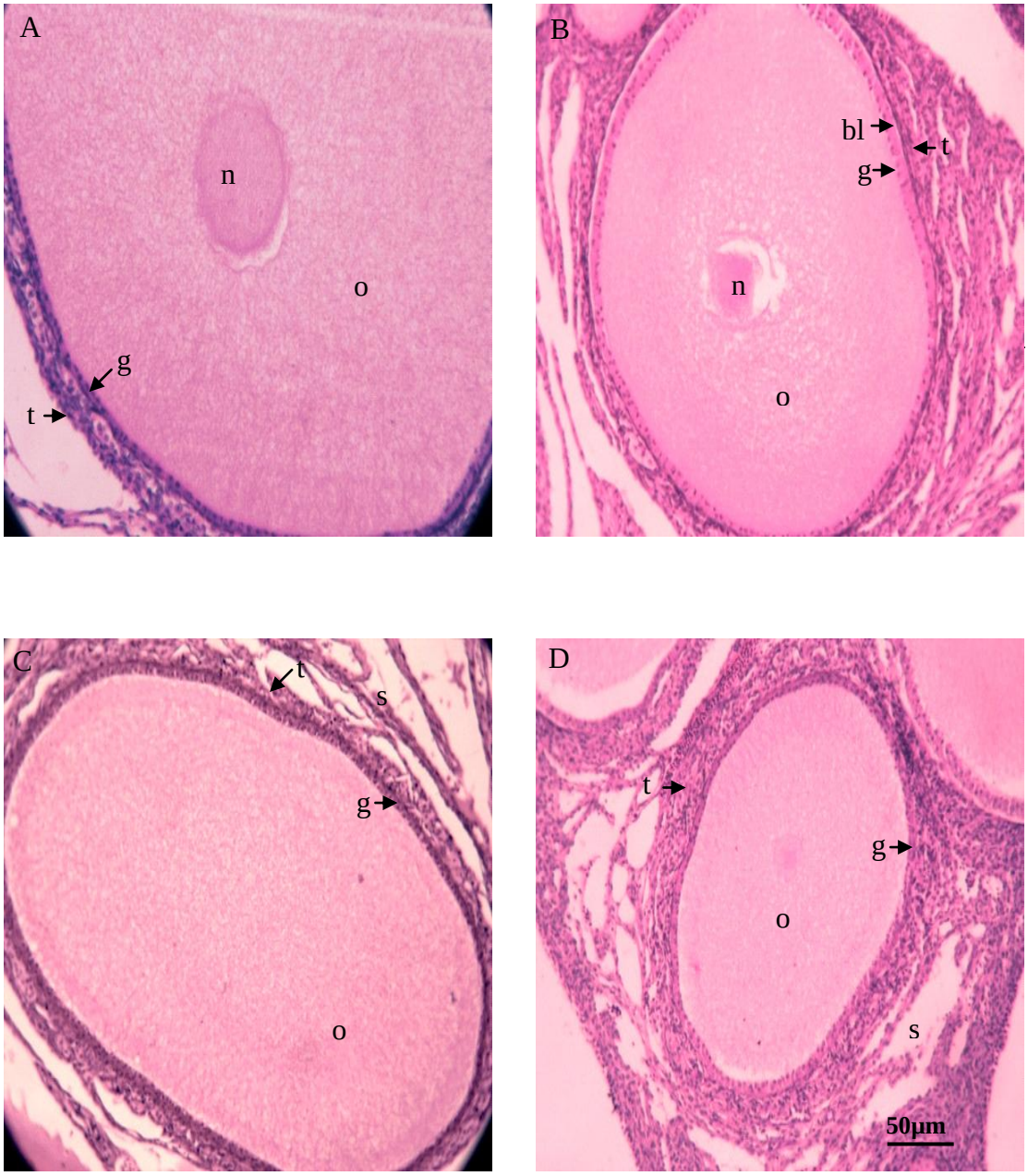


Fig 25: Photomicrograph of the middle portion of the cross section of ovary showing zona pellucida in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal feed at 25th week of age (Batch-II) (A) Control showing normal zona pellucida attached with granulosa layer (o) oocyte, (t) thecal layer, (g) granulosa layer, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing normal zona pellucida attached with granulosa layer (o) oocyte, (t) thecal layer, (g) granulosa layer, (n) nucleus and (bl) basal lamina. H & E.

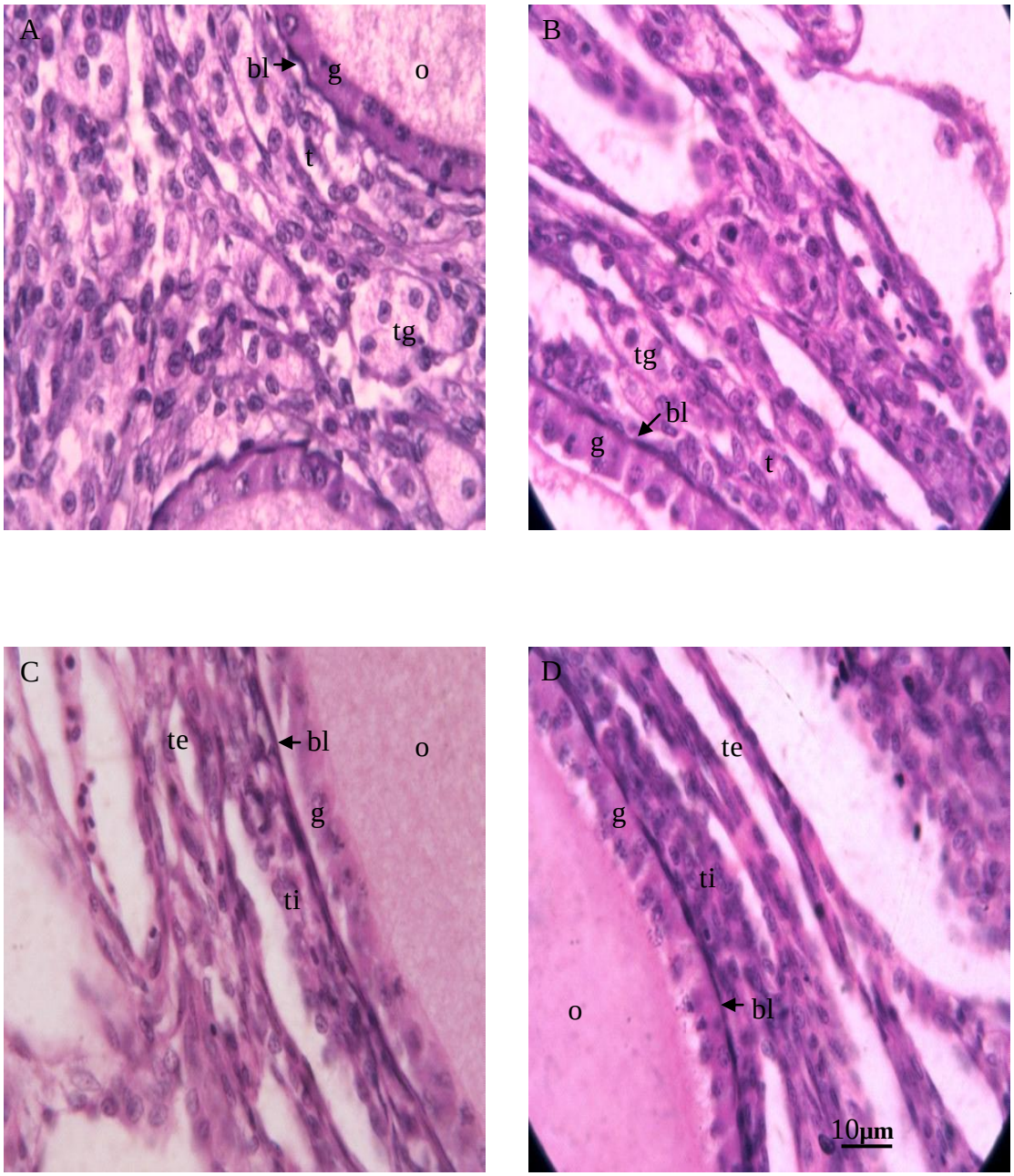


Fig 26: Photomicrograph of the middle portion of the cross section of ovary showing granulosa and basal lamina in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 25th week of age (Batch-II). (A) Control clear granulosa with basal lamina and thecal layer, (t) thecal layer, (g) granulosa, (bl) basal lamina, (tg) thecal gland, (o) oocyte (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing clear granulosa with basal lamina and thecal layer (ti) theca interna, (te) theca externa, (g) granulosa, (bl) basal lamina, (tg) thecal gland and (o) oocyte. H & E.

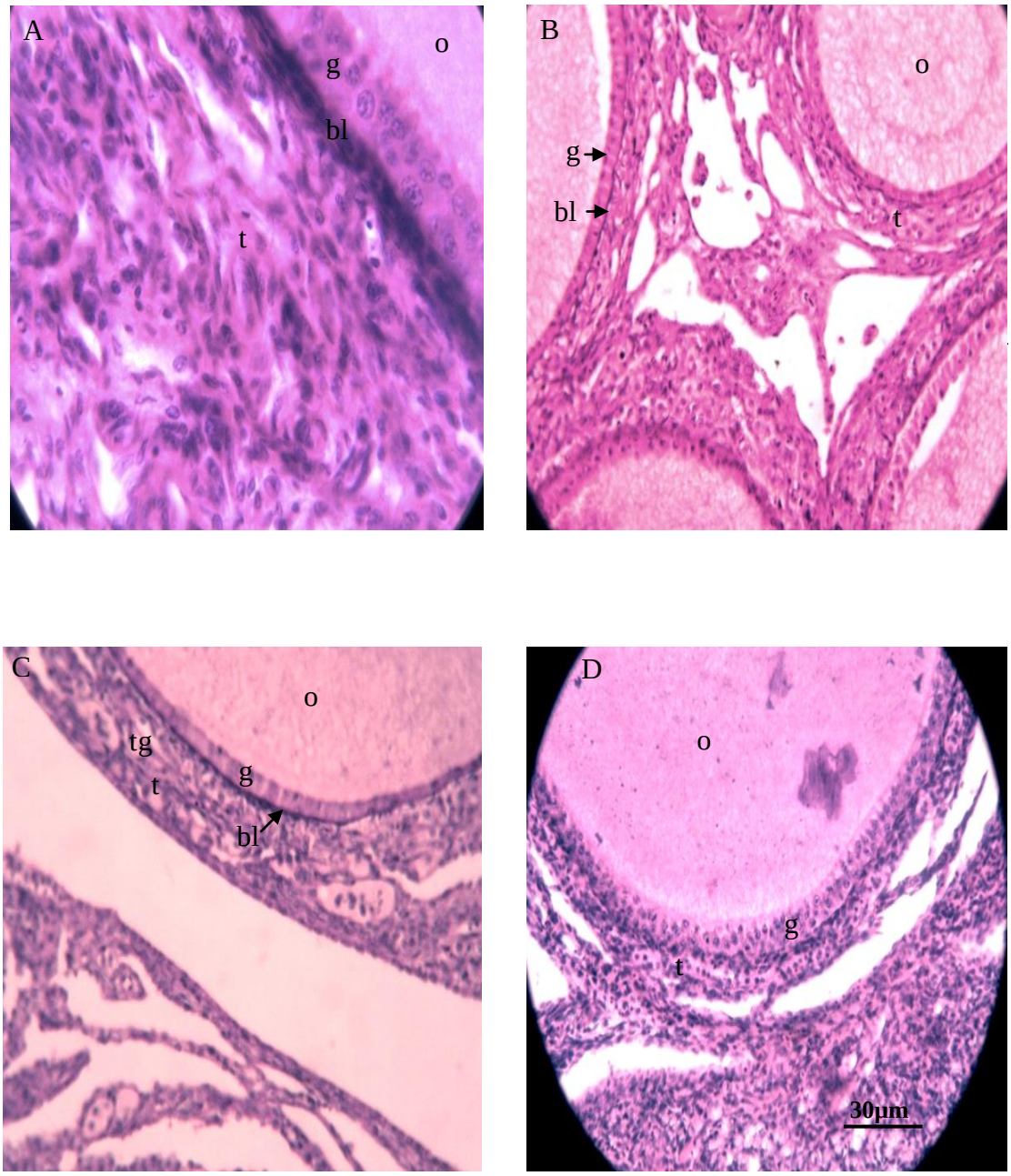


Fig 27: Photomicrograph of the middle portion of the cross section of ovary showing thickness of thecal layer in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 25th week of age (Batch-II). (A) Control showing greater thickness of follicular layer, (bl) basal lamina, (g) granulosa, (o) oocyte, (t) thecal layer, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing increased thickness of follicular layer (bl) basal lamina, (g) granulosa, (o) oocyte, (t) thecal layer and (tg) thecal gland. H & E.

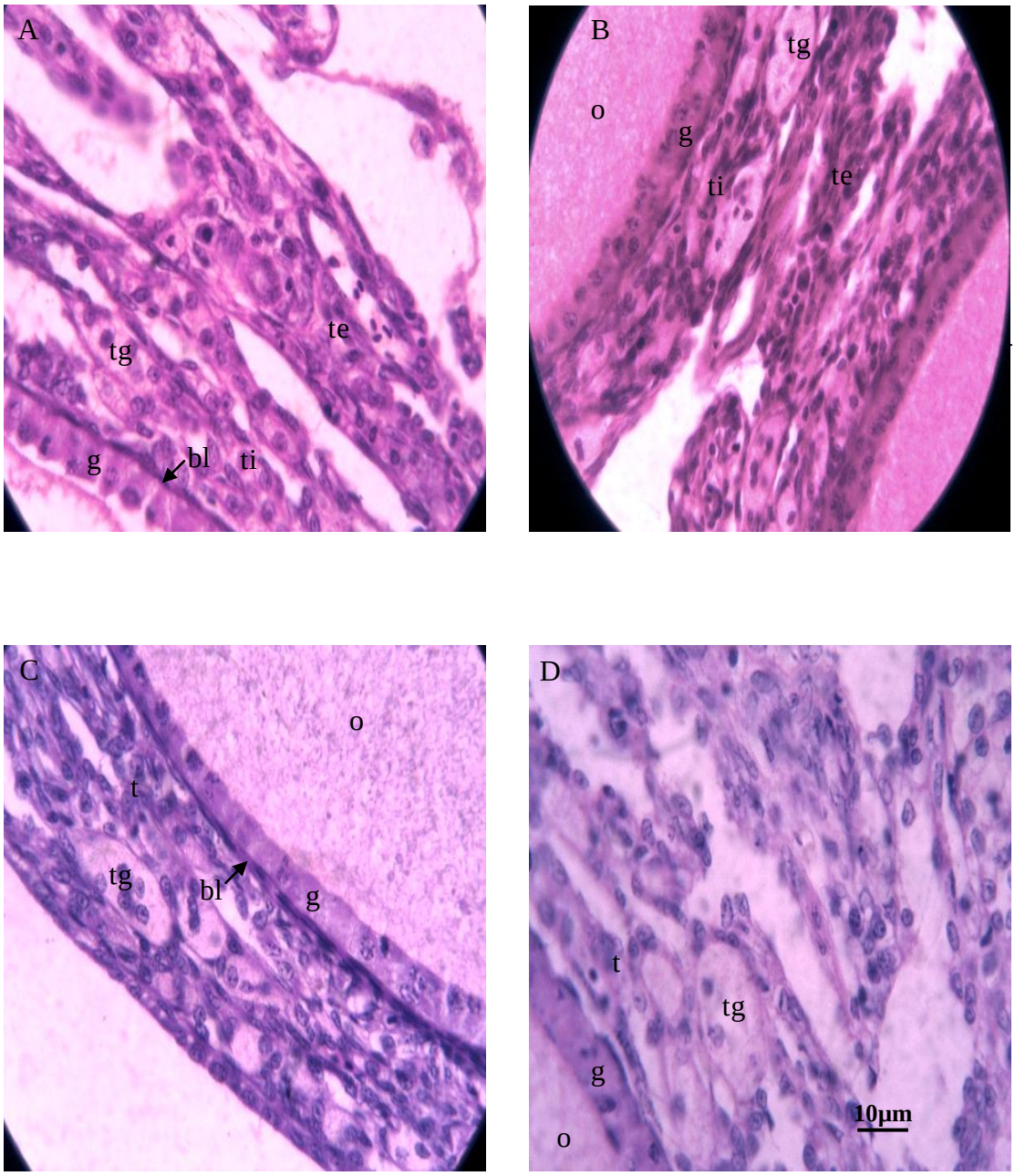


Fig 28: Photomicrograph of the middle portion of the cross section of ovary showing thecal gland concentration in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 25th week of age (Batch-II). (A) Control showing greater number of thecal gland in thecal layer, (tg) thecal gland, (g) granulosa, (o) oocyte, (ti) theca interna, (te) theca externa, (bl) basal lamina, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing increased number of thecal gland in thecal layer (tg) thecal gland, (g) granulosa, (o) oocyte, (t) thecal layer and (bl) basal lamina. H & E.

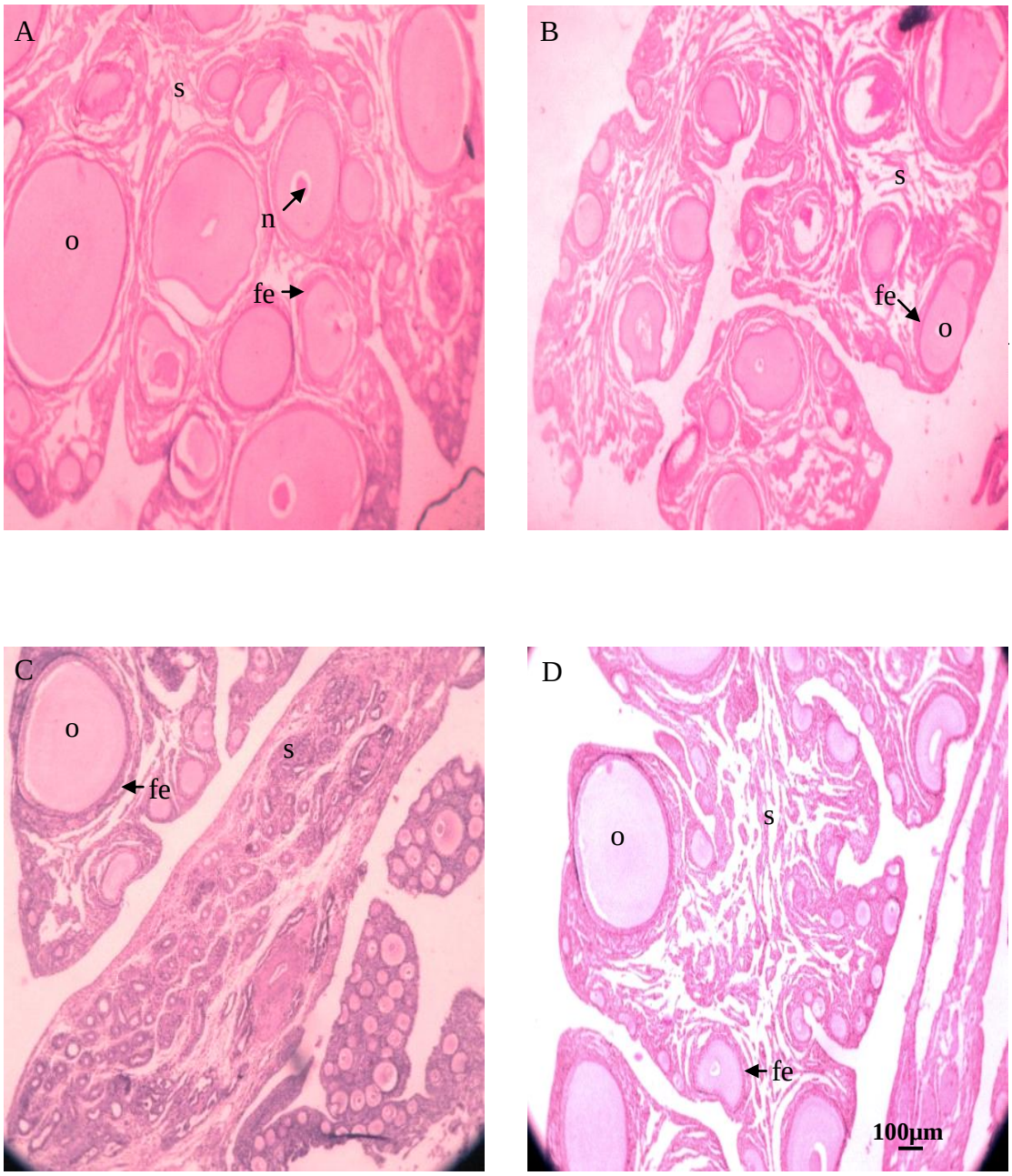


Fig 29: Photomicrograph of the middle portion of the cross section of ovary showing normal follicle in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 25th week of age (Batch-II). (A) Control showing no atretic follicle in follicle hierarchy, (fe) follicular epithelium, (s) stroma, (o) oocyte, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing no atretic follicle in follicle hierarchy (fe) follicular epithelium (s) stroma and (o) oocyte. H & E.

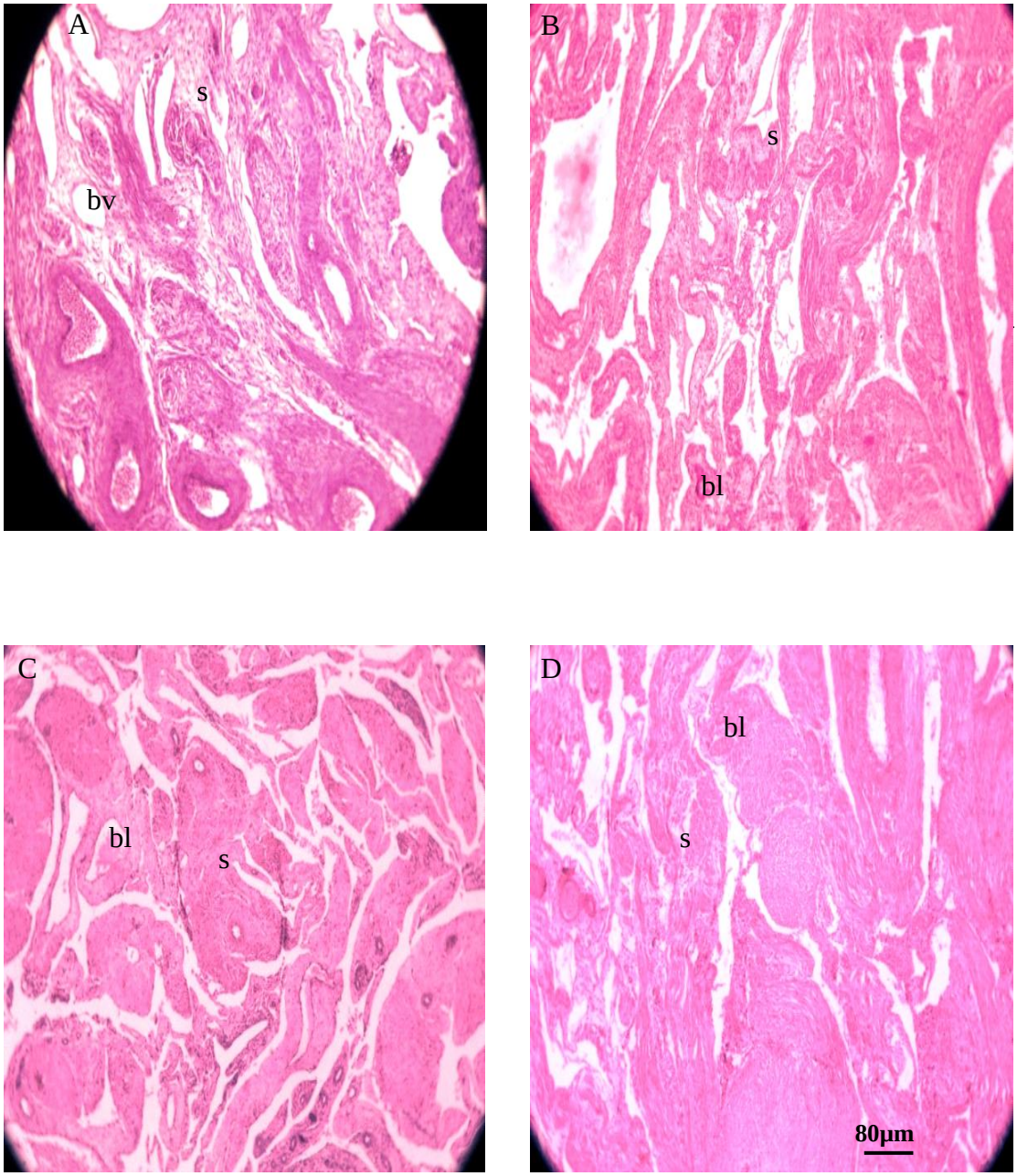


Fig 30: Photomicrograph of the middle portion of the cross section of ovary showing stromal tissue organization in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 25th week of age (Batch-II). (A) Control showing compact organization of stroma tissue with numerous blood vessels (bv) blood vessels, (s) stroma, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing compact organization of stromal tissue with numerous blood vessels (bv) blood vessels and (s) stroma. H & E.

EXPERIMENT-II

The experimental design for experiment-II was same as that of the experiment-I with the exception that the age of the chicks was 67 weeks. Birds were divided into four groups (N=10).

Group-I was fed normal feed and designated as control group.

Group-II birds were not fed for 12-days and kept on drinking water only

Group-III birds were treated with a dosage of 25,000ppm zinc/kg of feed for 12-days

Group-IV birds were treated with a dosage of 30,000ppm zinc/kg of feed for 12-days

The procedure followed was that after 12-days of treatment of 10 birds, five birds were slaughtered and observations were recorded as detailed in each group. Birds in all groups were provided water ad libitum. The slaughtered birds were designated as BATCH-I (12- DAYS BATCH).

The remaining five birds were fed on normal feed and slaughtered after 27-days from the start of experiment. The required observations were recorded, which are detailed in the respective groups. This part of the experiment was designated as BATCH-II (27-DAYS BATCH).

1 BODY WEIGHT

Body weight in each group was measured at an interval of two days and is presented in Table 28. At 12^{th} day of molting mean body weight was compared with control (Group-I) and within all groups (Fig. 31). Initial mean body weight in each group was also compared with the final weight at 12^{th} day of molting (Fig. 32).

Group-I (Control)

The chicks in the control group were fed normal diet during both segments of experiment. Increase in mean body weight was systematic and gradual. On 12^{th} day of experiment mean body weight was 1603±28.56gm which was significantly higher than the initial mean body weight ($t_{(18)}$=2.39; P=0.03). The linear regression analysis of variance shows significant increase in mean body weight with the advance in age (b=25.70±6.65; $F_{(1,3)}$=14.92; P=0.03)of birds.

Group-II (Off Fed)

The chicks were kept without feed for 12-days. A gradual decrease in the mean body weight was observed. The initial mean body weight was 1526.00±47.92gm and on 12^{th} day of molting it was 1081.50±47.11gm. This decrease in body weight on 12^{th} day was highly significant ($t_{(18)}$=6.62; P=0.001) compared to initial mean body weight. The regression analysis of variance showed that with the passage of time the decrease in mean body weight was highly significant (b=-107.90±16.82; $F_{(1,3)}$=41.15; P=0.008).

Group-III (25,000ppm Zinc/Kg Feed)

Ten birds were fed with feed mixed with zinc with a dosage of 25,000ppm for twelve days. At the start of experiment mean body weight of birds was 1478.50±25.36gm. On 12^{th} day of molting mean body weight was 1262±47.70gm. The decrease in body weight over the 12-days was highly significant ($t_{(18)}$=4.01: P=0.001) compared to that on the beginning of experiment.

The linear regression analysis of variance showed that with the passage of time there was highly significant decrease in mean body weight due to the effect of zinc mixed with normal feed (b=-51.70±12.96; $F_{(1,3)}$=15.91; P=0.03).

Group-IV (30,000ppm Zinc/Kg Feed)

Zinc with a dosage of 30,000ppm was mixed with feed and all birds were fed on this feed for a period of 12 days. Initial mean weight of the ten birds was 1514.00±38.24gm. On 12^{th} day of molting mean body weight was 1323.50±54.79gm. The difference between the two mean weights showed significant decrease ($t_{(18)}$=2.85; P=0.01). In Group-III there was 12% decrease in mean body weight after 12 days where as in Group-IV after 12 day there was 13% decrease in mean body weight.

Regression analysis of variance shows that due to mixing of zinc with normal feed and progression of time there was highly significant decrease in mean body weight (b=-48.05±11.66; $F_{(1,3)}$=16.99; P=0.03).

Comparisons

Mean body weight gain/loss on 12th day were compared among the four Groups. Compared to Control Group, Group-II ($t_{(18)}$=9.46; P=0.001), Group-III (Zinc treatment with 25,000ppm) ($t_{(18)}$=6.13; P=0.001) and Group-IV (Zinc treatment with 30,000ppm) ($t_{(18)}$=4.52; P=0.0003) showed highly significant decrease in mean body weight on 12th day. There was significant decrease in mean body weight in Group-II (off fed) compared to zinc treated Group-III ($t_{(18)}$=2.69; P=0.02) and Group-IV ($t_{(18)}$=3.35; P=0.004), but there was no significant difference in mean body weight in comparison between Group-III and Group-IV ($t_{(18)}$=0.85; P=0.41).

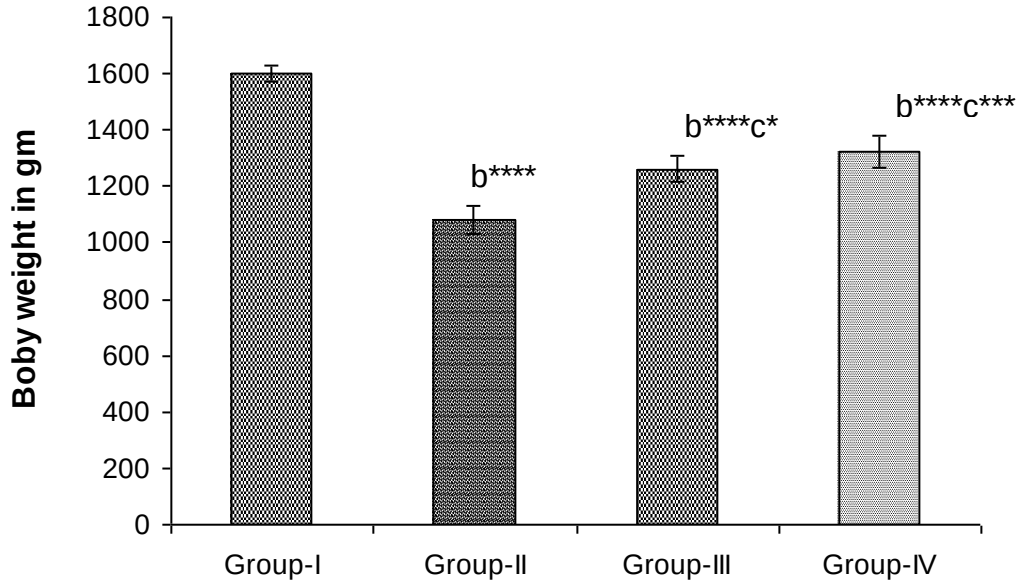


Fig. 31: Effect of restraint feeding and zinc administration on mean body weight (gm) in control and treated groups on 12th day of experiment at 67th week of age White Leghorn (WLH) layer birds (N=10).
b=Group-II compared to Group-III and group-IV at 12th day
c=Group-III compared to Group-IV at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

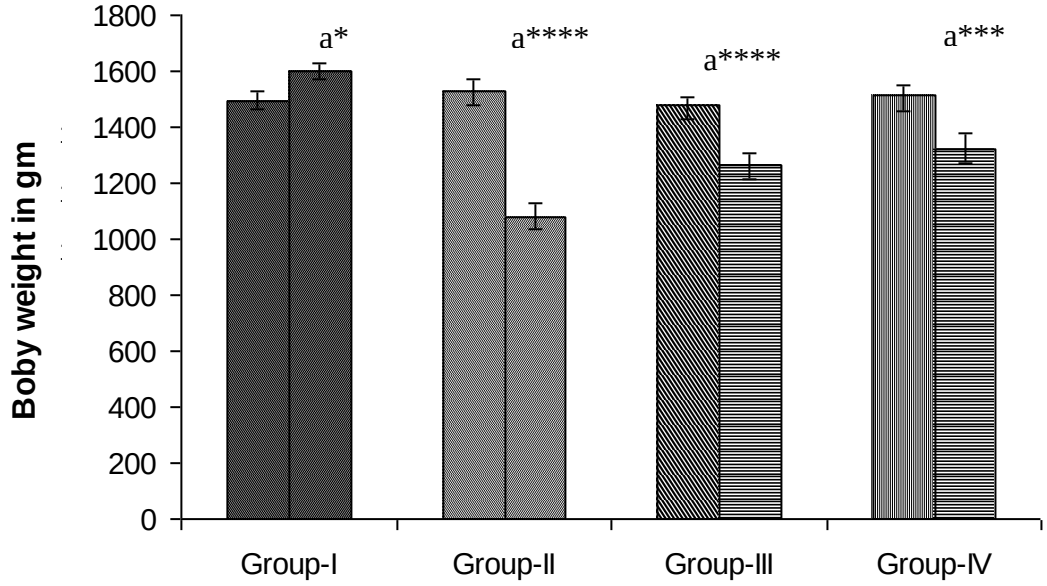


Fig. 32: Comparison of initial mean body weight and body weight (gm) on 12th day of restraint feeding and zinc administration in control and treated groups at 67th week of age White Leghorn (WLH) layer birds (N=10).
a=Group-I compared to all other groups at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

BATCH-I

1. BODY WEIGHT

Body weight in each group was measured at an interval of two days and is presented in Table 29. At the 12th day of molting mean body weight of all treatment groups was compared with control (Group-I) and within all groups (Fig. 33). Initial mean body weight in each group was also compared with the final weight at 12th day of molting (Fig. 34).

Group-I (Control Group)

At random five out of the ten chicks were slaughtered after 12 day of molting. Their initial mean body weight was 1477.00±41.40gm and on the 12th day it was 1550±27.20gm. The difference between the two was not significant ($t_{(8)}$=1.47; P=0.18). Regression analysis of variance indicated highly significant increase in mean body weight with the passage of time (b=16.60±2.32; $F_{(1,3)}$=51.28; P=0.006).

Group-II (Feed Restrained)

Initial body weight for the five slaughtered birds was 1430.00±58.57gm. These birds lost body weight which was 977.00±50.31gm on 12th day. This decrease in mean body weight on 12th day of molting was significant ($t_{(8)}$=5.87; P=0.0004). Over the period of 12 days there was significant loss of body weight (b=-110±16.92; $F_{(1,3)}$=42.24; P=0.007).

Group-III (25,000ppm Zinc/Kg Feed)

On 12th day of molting five of the ten birds were slaughtered randomly. Their initial mean body weight was 1429.00±18.62gm and on 12th day their mean weight was 1156.00±56.47gm. Compared to the initial weight, the decrease in body weight on 12th day of molting was significant ($t_{(8)}$=4.19; P=0.003). Regression analysis of variance also indicated significant decrease in mean body weight with the passage of time (b=-65.90±16.13; $F_{(1,3)}$=16.84; P=0.03).

Group-IV (30,000ppm Zinc/Kg Feed)

Five birds which were slaughtered on 12th day of molting their mean body weight was (1191.00±25.76gm) whereas their initial body weight was 1442.00±20.29gm. This

showed highly significant decrease ($t_{(8)}$=6.99: P=0.0001) in mean body weight compared to initial mean body weight. The linear Regression analysis of variance also showed significant decrease in mean body weight over the 12 days period (b=-69.00±16.13; $F_{(1,3)}$=18.29; P=0.026).

Comparisons

Comparisons of changes in mean body weight in different groups were also made to find out if they show the same trend as was seen with all the ten chickens. On the 12th day of molting, there was gain in mean body weight of Group-I (Control). Group-II ($t_{(8)}$=10.02; P<0.0001), Group-III (zinc dosage 25,000ppm); ($t_{(8)}$=5.83; P=0.0004) and Group-IV (zinc dosage 30,000ppm) ($t_{(8)}$=9.16; P<0.001) showed highly significant decrease in mean body weight compared to Group-I (control) but decrease in Group-II was greater compared to zinc treated groups. Within the groups comparisons, Group-II (Off fed) showed non-significant reduction ($t_{(8)}$=2.25; P=0.06) in mean body weight compared to Group-III (zinc dosage, 25,000ppm) however, Group-II exhibited significant lower mean body weight compared to Group-IV at the end of treatment ($t_{(8)}$=3.71; P=0.006). Group-III vs Group-IV ($t_{(8)}$=0.51; P=0.62) showed non-significant difference in mean body weight.

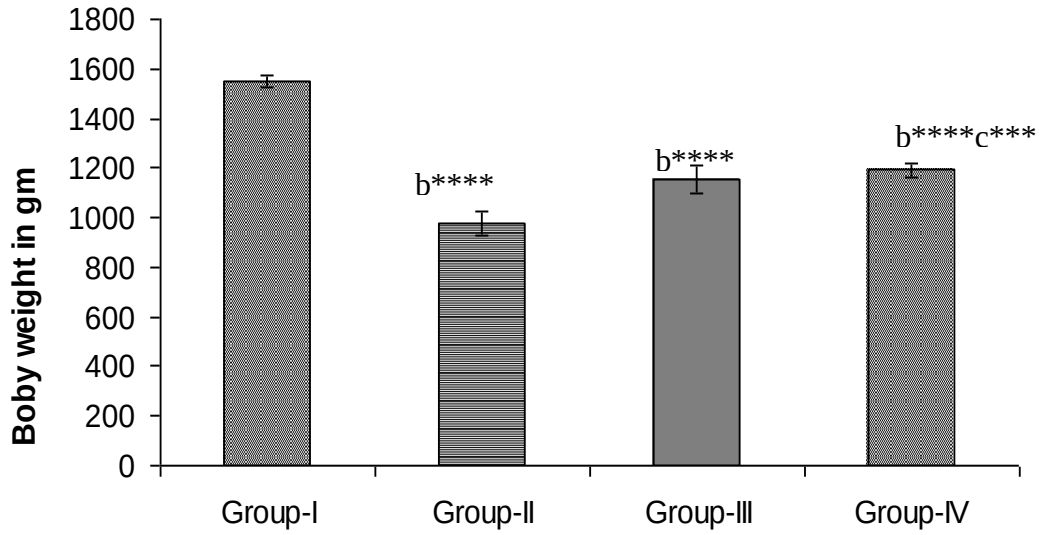


Fig. 33. Effect of restraint feeding and zinc administration on mean body weight (gm) in control and treated groups at 67th week of age White Leghorn (WLH) layer birds slaughtered on 12th day of experiment (Batch-I)
b=Group-II compared to Group-III and group-IV at 12th day
c=Group-III compared to Group-IV at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

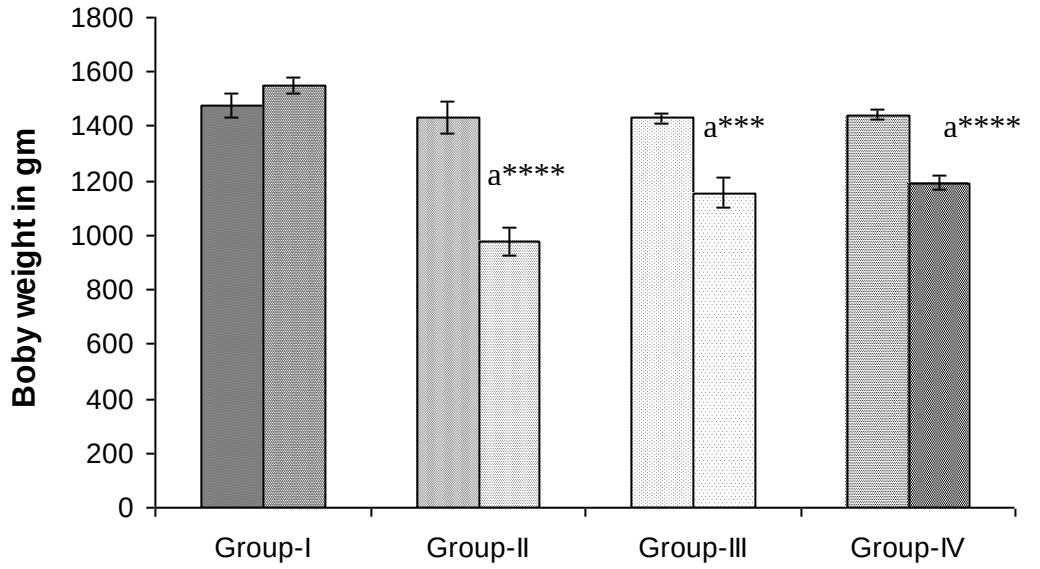


Fig 34: Comparison of initial body weight and body weight (gm) in birds slaughtered on 12th day of restraint feeding and zinc administration in control and treated groups at 67th week of age White Leghorn (WLH) layer birds.(Batch-I)
a=Group-I compared to all other groups at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

2 SECONDARY SEXUAL CHARACTERISTICS

Comb and Wattles

Morphometric measurement of wattle and comb were recorded for Batch-I to find out changes occurring in maintaining birds with completely restrained feed and feed mixed with zinc. Mean length and width of comb and wattle in four groups are shown in Table 30.

Comb

There was highly significant decrease in comb length in Group-II ($t_{(8)}$=22.20; P<0.0001), Group-III ($t_{(8)}$=2.86; P=0.02) and Group-IV ($t_{(8)}$=3.78; P=0.005) compared to the Group-I (control group). In Group-II mean comb length was highly significantly lowest compared to Group-III ($t_{(8)}$=11.65; P<0.0001) and Group-IV ($t_{(8)}$=27.02; P<0.0001). Similarly, significant reduction in comb width was observed in Group-II ($t_{(8)}$=21.92; P<0.001) and Group-IV ($t_{(8)}$=3.06; P=0.02) compared to Group-I (control) but the reduction in Group-III ($t_{(8)}$=1.44; P=0.19) was non-significant. Comb width in Group-II (off fed) was significantly lower compared to Group-III ($t_{(8)}$=4.09; P=0.002) and Group-IV ($t_{(8)}$=8.58; P<0.0001). However, there was no significant difference in comb width in a comparison between Group-III Vs Group-IV ($t_{(8)}$=0.20; P=0.84).

Wattles

In Batch-I which was slaughtered after 12 days of molting, significant reduction was noticed in mean wattles length in Group II ($t_{(8)}$=11.45; P<0.0001) Group-III ($t_{(8)}$=4.11; P=0.003) and Group-IV ($t_{(8)}$=2.69; P=0.03) compared to Group-I (control). There was no significant difference in wattles mean length in Group-III and Group-IV compared to Group-II (off fed). Wattles length in Group-III Vs Group-IV also remained unchanged.

Mean wattles width in Group-II ($t_{(8)}$=2.55; P=0.03) and Group-IV ($t_{(8)}$=2.38; P=0.04) was significantly reduced while, wattles width in Group-III (25,000ppm Zn) ($t_{(8)}$=1.12; P=0.29) decreased non significantly compared to that of Group-I. When wattles width of Group-II was compared to Group-III ($t_{(8)}$=1.36; P=0.21) and Group-IV ($t_{(8)}$=0.53; P=0.61) it showed non significant variation in wattles width. There was also no significant difference in wattles width ($t_{(8)}$=1.08; P=0.31) in a comparison between Group-III Vs Group-IV.

3. EFFECT ON OVARY, OVIDUCT AND LIVER

Ovarian weight, length and width, oviduct weight and width and liver weight were recorded after slaughtering the chicks and are shown in Table 31.

Mean ovarian weight reduced in all treatment groups. There was highly significant reduction of ovarian weight in Group-II ($t_{(8)}$=25.53; P<0.0001), Group-III ($t_{(8)}$=14.83; P<0.0001) and Group-IV ($t_{(8)}$=23.36; P<0.0001) compared to Group-I (control). Reduction in ovarian weight of Group-II (off fed) was also significant when compared to zinc treated Group-III ($t_{(8)}$=2.85; P=0.02) and Group-IV ($t_{(8)}$=3.69; P=0.006). Group-III vs Group-IV did not show significant ($t_{(8)}$=0.24; P=1.27) difference between them.

There was significant reduction in mean ovarian length in Group-II ($t_{(8)}$=3.13; P=0.01), Group-III ($t_{(8)}$=2.82; P=0.02) and in Group-IV ($t_{(8)}$=2.61; P=0.03) compared to Group-I. No significant difference in ovarian length was noticed in comparison of Group-II (off fed) vs zinc treated Group-III ($t_{(8)}$=0.71; P=0.49) and Group-IV ($t_{(8)}$=0.84; P=0.42). Same trend was seen in comparison between Group-III Vs Group-IV ($t_{(8)}$=0.19; P=0.85).

Ovarian width decreased non significantly in Group-II ($t_{(8)}$=1.66; P=0.13), Group-III ($t_{(8)}$=2.01; P=0.08) and Group-IV ($t_{(8)}$=1.36; P=0.21) compared to Group-I (control). Comparison among treatment groups also showed non significant difference in mean ovarian width.

There was highly significant reduction in mean oviductal weight in Group-II ($t_{(8)}$=19.06; P<0.0001), Group-III ($t_{(8)}$=9.74; P<0.0001) and Group-IV ($t_{(8)}$=13.94; P<0.0001) compared to Group-I (control) however, decrease was greatest in off fed group. Mean oviductal weight was significantly lower in Group-II compared to Group-IV ($t_{(8)}$=3.24; P=0.01) but Group-III did not differ significantly ($t_{(8)}$=1.91; P=0.09). No significant difference in Group-III vs Group-IV ($t_{(8)}$=0.20; P=0.98) was observed.

Mean oviductal length decreased significantly in Group-II ($t_{(8)}$=12.11; P<0.0001), Group-III ($t_{(8)}$=5.09; P=0.001) and in Group-IV ($t_{(8)}$=10.54; P<0.0001) compared to Group-I. There was significant difference in oviductal length in comparison of Group-II vs Group-III ($t_{(8)}$=2.57; P=0.03) but Group-IV ($t_{(8)}$=0.31; P=0.77) showed non

significant decrease. In Group-III vs Group-IV no significant change ($t_{(8)}$=2.25; P=0.054) was observed.

The liver weight in off fed birds Group-II ($t_{(8)}$=7.87; P<0.0001) as well as zinc treated Group-III (25,000ppm Zn) ($t_{(8)}$=2.95; P=0.02) and Group-IV (30,000ppm Zn) ($t_{(8)}$=3.24; P=0.01) showed significant decrease compared to Group-I (control) however, this decrease was greater in off fed group. Mean liver weight of Group-II was also significantly lower compared to Group-III ($t_{(8)}$=2.86; P=0.02) and Group-IV ($t_{(8)}$=5.35; P=0.002). There was no significant difference ($t_{(8)}$=0.28; P=0.78) in mean liver weight between Group-III vs Group-IV.

4. ZINC DEPOSITION IN DIFFERENT ORGANS TISSUE

Deposition of zinc in different organs (µg/gm) of Group-I (control), (Group-II) restrained feeding, zinc with 25,000ppm dosage (Group-III) and zinc with 30,000ppm dosage (Group-IV) is given in Table 32.

Deposition of Zinc in Ovary

There was no appreciable difference in zinc deposition in the ovary between Group-I and Group-II ($t_{(8)}$=0.05; P=0.96) birds. However, birds treated with 25,000ppm zinc dosage (Group-III) ($t_{(8)}$=5.33; P=0.001) and those treated with 30,000ppm dosage (Group-IV) ($t_{(8)}$=5.69; P=0.0005) showed significantly higher zinc deposition compared to Group-I (control). Highly significant increase in mean zinc deposition in Group-III ($t_{(8)}$=5.25; P=0.0008) and Group-IV ($t_{(8)}$=5.67; P=0.0005) was observed compared to Group-II however, increase was highest in the Group-IV. The group which was treated with high dose of zinc (Group-IV) showed significantly ($t_{(8)}$=2.51; P=0.04) higher zinc deposition compared to Group-III (25,000ppm zinc/Kg feed).

Deposition Of Zinc In Liver

Mean zinc deposition in the liver of molted birds of Group-II (off fed) ($t_{(8)}$=6.91; P=0.0001), Group-III (25,000ppm zinc) ($t_{(8)}$=8.27; P<0.0001) and Group-IV (30,000ppm zinc) ($t_{(8)}$=21.47; P<0.0001) showed significantly higher concentration compared to Group-I (control). In this Batch-I, there was highly significant increase in zinc deposition in Group-III (25,000ppm dosage) ($t_{(8)}$=10.41; P<0.0001) and Group-

IV (30,000ppm dosage) ($t_{(8)}$=23.91; P<0.0001) compared to Group-II. There was also very high zinc deposition in Group-IV compared to Group-III ($t_{(8)}$=9.85; P<0.0001).

Deposition of Zinc in Kidneys

In kidneys there was no significant difference in zinc deposition between Group-I and Group-II ($t_{(8)}$=0.78; P=0.46) birds. However, birds treated with 25,000ppm zinc dosage (Group-III) ($t_{(8)}$=7.01; P=0.0001) and those treated with 30,000ppm dosage (Group-IV) ($t_{(8)}$=10.05; P<0.0001) showed significantly higher zinc deposition compared to Group-I (control). In Group-III ($t_{(8)}$=7.82; P<0.0001) and Group-IV ($t_{(8)}$=11.01; P<0.0001) significantly high zinc deposition was seen compared to Group-II (off fed). In comparison with Group-III vs Group-IV a significant increase in zinc deposition ($t_{(8)}$=2.45; P=0.04) was seen in Group-IV.

Table 32: Mean Zinc Concentration (µg/g) in ovary, kidney and liver in White Leghorn (WLH) layer birds at 67th week of age slaughtered after 12th day of experiment (Batch-I)

Treatment groups	During the administration of Zinc oxide		
	Ovary	**Liver**	**Kidney**
Group I	15.56±0.60	5.95±0.47	9.10±0.72
Group-II	14.11±0.80	13.42±0.90^{a****}	13.34±1.04
Group-III	19.35±1.85^{ab****}	37.29±3.86^{ab****}	35.93±2.69^{ab****}
Group-IV	30.25±3.51^{ab****c*}	81.51±5.35^{abc****}	45.30±2.71^{ab****c*}

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

5 HORMONAL ESTIMATION

Plasma Estradiol Level

The concentration of estradiol in the plasma of control and different treatment groups during the treatment period, as well as at the end of experiment on 12 day is shown in Table 33. On 3rd day of molt induction, the hens in Group-II, Group-III and Group-IV have significantly lower ($t_{(18)}$=9.30; P<0.0001, $t_{(18)}$=8.21; P<0.0001 and $t_{(18)}$=8.11; P<0.0001) level of estradiol in their plasma compared to the levels in Group-I (control). The plasma level of estradiol in Group-II ($t_{(18)}$=10.47; P<0.0001), Group-III ($t_{(18)}$=9.04; P<0.0001) and Group-IV ($t_{(18)}$=10.23; P<0.0001) were significantly lower than Group-I (control) on 6th day of treatment. This reduction in mean estradiol level continued in all the treatment groups on 9th day in Group-II ($t_{(18)}$=29.51; P<0.0001), Group-III ($t_{(18)}$=19.73; P<0.0001) and Group-IV ($t_{(18)}$=22.15; P<0.0001). On 12th day of experiment decrease in plasma estradiol was more evident in all treatment groups which include Group-II ($t_{(18)}$=16.26; P<0.0001), Group-III ($t_{(18)}$=18.79; P<0.0001) and Group-IV ($t_{(18)}$=18.91; P<0.0001) compared to Group-I (control). There was no significant difference in the estradiol levels of the among different treatment groups when they were compared to one other on day 3, 6, 9 and 12.

Linear regression analysis of variance showed that there was no significant change in plasma estradiol concentration with the advance in days (b=1.66±2.37; $F_{(1,3)}$=0.49; P=0.53) in control (Group-I), but there was highly significant decrease in plasma estradiol concentration in Group-II (off fed) (b=-39.92±10.59; $F_{(1,3)}$=14.21; P=0.033), as well as Zn treated Group-III (25,000ppm/Kg) (b=-44.01±11.04; $F_{(1,3)}$=15.90; P=0.03) and Group-IV (30,000ppm/Kg) (b=-41.77±12.54; $F_{(1,3)}$=11.09; P=0.05) with the advance in the days of molting (Fig. 35).

Plasma Progesterone Level

The mean concentration of progesterone in the plasma with different treatments is shown in Table 34. There was highly significant reduction in mean progesterone concentration in all induced molt groups, Group-II ($t_{(18)}$=25.53; P<0.0001), Group-III ($t_{(18)}$=19.46; P<0.0001) and Group-IV ($t_{(18)}$=24.57; P<0.0001) compared to Group-I (control) on 3rd day of experiment. On 6th day of molt induction same trend of reduction in progesterone level in all the treatment groups i.e. Group-II ($t_{(18)}$=15.80;

P<0.0001), Group-III ($t_{(18)}$=18.49; P<0.0001) and Group-IV ($t_{(18)}$=15.29; P<0.0001) was observed compared to Group-I (control). On day nine (9) plasma level of progesterone was highly reduced in Group-II ($t_{(18)}$=8.47; P<0.0001), Group-III ($t_{(18)}$=8.35; P<0.0001), and Group-IV ($t_{(18)}$=8.40; P<0.0001) compared to Group-I (control). On 12th day of molting plasma progesterone level remained lower in Group-II ($t_{(18)}$=4.90; P=0.0001), Group-III ($t_{(18)}$=4.61; P=0.0002) and Group-IV ($t_{(18)}$=4.74; P=0.0002) compared to unmolted group (control).

Comparison among all treatment groups showed no significant difference in mean plasma progesterone level on 3rd, 6th , 9th and 12th day of experiment.

Linear regression analysis of variance showed that there was no significant change in plasma progesterone concentration in control (Group-I) (b=0.05±0.08; $F_{(1,3)}$=0.44; P=0.56), whereas there was highly significant decrease in plasma progesterone concentration in Group-II (off fed) (b=-0.74±0.20; $F_{(1,3)}$=14.10; P=0.03), as well as Zn treated Group-III (25,000ppm/Kg) (b=-0.66±0.20; $F_{(1,3)}$=10.60; P=0.047) and Group-IV (30,000ppm/Kg) (b=-0.73±0.20; $F_{(1,3)}$=13.15; P=0.04) with the advance in the days of treatment (Fig. 36).

Plasma Corticosterone Level

The mean plasma concentration of corticosterone in the different treatments is presented in Table 35. On 3rd day of molting mean plasma corticosterone level started increasing significantly in Group-II ($t_{(18)}$=4.34; P=0.0004), Group-III ($t_{(18)}$=4.24; P=0.0005) and Group-IV ($t_{(18)}$=11.89; P<0.0001) compared to Group-I (control) but the elevation was more evident in Group-II (off fed). Corticosterone level on 6th day of molting revealed highly significant increase in Group-II ($t_{(18)}$=4.59; P=0.0002), Group-III ($t_{(18)}$=8.05; P<0.0001) and Group-IV ($t_{(18)}$=8.21; P<0.0001) compared to Group-I (control). On 9th day of experiment mean corticosterone concentration of Group-II ($t_{(18)}$=17.54; P<0.0001), Group-III ($t_{(18)}$=25.75; P<0.0001) and Group-IV ($t_{(18)}$=20.77; P<0.0001) showed highly significant escalation compared to Group-I (control). Highly significant increase in corticosterone level was also observed in Group-II ($t_{(18)}$=33.44; P<0.0001), Group-III ($t_{(18)}$=27.63; P<0.0001) and Group-IV ($t_{(18)}$=27.44; P<0.0001) compared to Group-I (control) on 12th day of experiment. Comparison of mean plasma corticosterone concentration showed no significant difference among all treatment groups throughout the experiment starting from day 1-12.

Linear regression analysis of variance showed that there was no significant change in plasma corticosterone concentration with the advance in days of treatment (b=-0.11±0.37; $F_{(1,3)}$=0.09; P=0.78) in control (Group-I), but non significant increase in concentration in plasma, Group-II (off fed) (b=6.28±4.25; $F_{(1,3)}$=2.19; P=0.24), Group-III (25,000ppm/Kg) (b=6.86±3.75; $F_{(1,3)}$=3.36; P=0.16) and Group-IV (30,000ppm/Kg) (b=6.76±2.81; $F_{(1,3)}$=5.81; P=0.09) with the advancement in the days of treatment (Fig. 37).

6 MORPHOMETRY

Mean Ovarian Yolky Follicles Numbers

Mean number of yolky follicles in control and treated groups is presented in Table 36. There was highly significant reduction ($P<0.001$) in the mean number of yolky follicles in Group-II (off fed), Group-III (25,000ppm zinc/Kg feed) and Group-IV (30,000ppm zinc/Kg feed) compared to Group-I in 1-5mm Category of yolky follicles. Group-III vs Group-IV comparison showed no significant difference. One way analysis of variance showed highly significant differences in mean number of yolky follicles in different groups $F_{(3,16)}=19.50$; $P<0.001$. Category 5.1-10mm showed significant decrease ($P<0.001$) in mean yolky follicles number in Group-II and Group-IV but Group-III did not differ significantly compared to Group-I. In categories 10.1-15mm, 15.1-20mm, 20.1-25mm and 25.1-30mm comparison can not be calculated because numbers of follicles observed are statistically insufficient in all treated groups.

Mean Ovarian Yolky Follicles Diameter

In Batch-I mean diameter of yolky follicles was recorded and arranged in different categories Table 37.

In yolky follicle category 1-5mm significant reduction in mean diameter in Group-II and Group-IV ($P<0.02$) was observed, but Group-III did not differ significantly compared to Group-I. In comparison of Group-II vs Group-III significant decrease ($P<0.05$) was observed in yolky follicle diameter in Group-II. Category 5.1-10mm showed significant decrease ($P<0.05$) in mean diameter of yolky follicles in Group-IV while Group-II and Group-III did not differ significantly compared to Group-I. In this category mean follicle diameter in Group-IV was significantly lesser ($P<0.05$) compared to Group-II. In categories 10.1-15mm, 15.1-20mm, 20.1-25mm, 25.1-30mm and <30 mm of treatment groups either one or two yolky follicles were recorded or they did not develop in these categories.

Mean Non Yolky Ovarian Follicular Diameter

Ovarian non yolky follicular diameter was arranged in different categories in Table 38. In Group-III and Group-IV smallest follicular diameter (≤200µm) reduced highly significantly compared to Group-I ($t_{(112)}$=2.16; P=0.03; $t_{(64)}$=2.04; P=0.05 respectively). In follicular diameter category 201-400µm, 401-600µm, 601-800µm no significant difference was observed in Group-II, Group-III and Group-IV compared to that of Group-I. In follicular categories 801-1000µm and >1001µm the follicles did not develop n Group-II and Group-IV This could be due to off fed condition and high dose of zinc (30,000ppm zinc/Kg feed) that their development was arrested.

Mean Non Yolky Oocyte Diameter

In this experiment the treated chicks were slaughtered on 12th day (at 67 weeks of age) after starting the experiment (Batch-I). Mean non yolky oocyte diameter in different categories is shown in Table 39. As in Experiment-I the same experimental design was followed (i.e. Group-I (control), Group-II (off fed), Group-III (25,000ppm zinc/Kg feed) and Group-IV (30,000ppm zinc/Kg feed)). Mean oocyte diameter in Group-II showed significant decrease compared to Group-I in category ≤200µm ($t_{(52)}$=2.09; P=0.04). In comparison, Group-II vs Group-III significant decrease ($t_{(72)}$=2.50; P=0.01) was observed in oocyte diameter in Group-II. In categories 201-400µm, 401-600µm and 601-800µm, there was no significant difference in mean oocyte diameter of Group-II, Group-III and Group-IV compared to Group-I. In Group-II and Group-IV since there were no follicles in categories 801-1000µm and >1001µm hence no oocyte diameter was measured. This may be the result of off fed condition and high dose of zinc (30,000ppm zinc/Kg feed) which arrested the development of follicles and their oocytes as well.

Mean Non Yolky follicular wall thickness

Mean non yolky follicular wall thickness in different categories in the four Groups of treatment is given in Table 40. Compared to control (Group-I), Group-II ($t_{(52)}$=6.92; P<0.0001), Group-III ($t_{(112)}$=5.07; P<0.0001) and Group-IV ($t_{(64)}$=5.06; P<0.0001) showed highly significant reduction in mean thickness of follicular wall in smallest category (≤20µm). Follicular wall thickness in category 21-40µm showed significant reduction in thickness in Group-II ($t_{(115)}$=2.19; P=0.03) compared to Group-I (control) while, in other groups there was no change. Follicular wall thickness in category 41-60µm, compared to control showed significant reduction in follicular wall thickness in Group-II ($t_{(89)}$=5.37; P<0.0001), Group-III ($t_{(158)}$=4.90; P<0.0001) and Group-IV ($t_{(100)}$=3.94; P=0.0002). Similarly, Group-II vs Group-III ($t_{(97)}$=2.50; P=0.01) showed significant reduction in mean follicular wall thickness in category 41-60µm. The follicular wall thickness category 61-80µm showed no significant difference in mean follicular wall thickness in all treatment groups compared to control group. The follicular wall thick in the categories 80-100µm and >101µm showed no significant difference in Group-II compared to Group-I. In Group-III and Group-IV no follicles were present hence no follicular wall thickness was measured in these categories.

Mean Non Yolky Ovarian Follicular Number

Mean number of non yolky ovarian follicles in different categories in the four groups is shown in Table 41. Group-II ($t_{(8)}$=2.72; P=0.02) and Group-IV ($t_{(8)}$=2.74; P=0.02) compared to Group-I showed highly significant decrease in mean number of follicles in category ≤200µm. In category 201-400µm significant decrease in follicle number was seen in Group-II (off fed) ($t_{(8)}$=2.63; P=0.03) compared to Group-I. In category 401-600µm**,** there was no significant difference in mean follicle number in all treatment groups compared to Group-I. As in Batch-I of experiment-I in control group follicles categories 601-800µm 801-1000µm and >1001µm were present. These categories of follicles were not present in Group-II (off fed) and high dose of zinc Group-IV. In Group-III only 601-800µm category follicles were present, while 801-1000µm and >1000µm did not develop. but in Group-II, Group-III and Group-IV did not developed these categories. Follicle in category 601-800µm were seen in Group-III.

7 HISTOMORPHOLOGY

General and Histological Observations of Ovaries

Ovarian Weight and size in off fed, low and high zinc dose treatment groups decreased during treatment period compared to Group-I (Table 31). After slaughtering the birds their ovaries were removed. Large yolky and small whitish (transparent) yolky follicles were counted and measured (Table 36 and 37). In control birds ovaries more yolky follicles were observed compared to treated birds. Blood vessels were visible in larger yellow yolky follicles surrounded by vitelline membrane and suture line or stigma was also very clear in these follicles. Study of cross section of middle portion of the White Leghorn ovary showed that it possessed two distinct regions, an outer cortex and inner medulla. The cortex contained follicles of variable size range. In between the follicles abundant compactly arranged stromal tissue was present. The inner medullar stroma was composed of well vascularised and innervated connective tissue with spaces (lacunae), nerves and blood vessels.

The control group ovaries contained number of healthy non yolky follicle at various developmental stages. Number of ovarian follicles decreased in Group-II (off fed) (Fig. 38B) and zinc treated Group-III and Group-IV (Fig. 38CD) compared to control group (Fig. 38A). In control group follicles of all diameter categories from ≤200µm to >1001µm were present whereas high dose treatment group (30,000ppm zinc/Kg feed) and off fed group larger categories from 601-800µm to >1001µm follicles did not develop. Number and diameter of follicles in category ≤200µm also decreased in off fed (Fig. 38B) and high dose treatment groups (30,000ppm zinc/Kg feed) (Fig. 38CD).

In control group, histologically large follicle contained well defined spherical nucleus with distinct network of chromatin and clear nuclear membrane. The ooplasm of the follicle was covered with zona pellucida. The oocyte contained ooplasm and was surrounded by zona pellucida. Tiny cytoplasmic extension from the granulosa passed through the zona pellucid connecting them to the oocyte. The granulosa layer of follicles is composed of cuboidal cells around the oocyte. Above the granulosa layer basal lamina layer separated the granulosa layer from follicular epithelium

(thecal layer). Thecal layer was distinguishable into two layers, i.e. theca interna and theca externa. There were numerous thecal glands in theca interna for production of steroid hormones for stimulation of folliculogenesis and ovulation processes.

Oocytes of ovarian follicles in the control group contained well defined elliptical nucleus with distinct network of chromatin and clear nuclear membrane (Fig. 39A). While in off fed group and zinc treated bird groups, the oocyte nucleus was not well defined and nuclear matrix exhibited shrink appearance (Fig. 39BCD).

There was no difference in structure of primordial follicles in treated groups compared to control (Fig. 40A). In off fed (Fig. 40B) and both zinc treated groups ovaries possessed larger follicles with abnormal oocytes and disrupted ooplasm (Fig. 40CD). In these oocyte the nucleus also detached from center and became irregular in shape lacking nuclear material.

The thin layer of zona pellucida between ooplasm and granulosa cells was disrupted in follicles of off fed group (Fig. 41B) and both zinc treated groups (Group-III and Group-IV) (Fig. 41CD). The zona pellucida layer detached and lacked the cytoplasmic processes into granulosa layer.

The granulosa layer of the follicles was composed of cuboidal cells i.e. basal lamina and granulosa layer. Granulosa layer and basal lamina in follicles of off fed (Fig. 42B) and both zinc treated groups were thick, intermingled with each other and no clear demarcation between them (Fig. 42CD) was seen whereas a clear demarcation was observed in control birds (Fig. 42A).

Reduction in thickness of peripheral follicular epithelial (theca interna and theca externa) layer was seen in all categories of follicles in off fed (Fig. 43B) and low and high dose zinc treated groups (Fig. 43CD) compared to control (Fig. 43A) . But the greater decrease in thickness of follicular epithelial layer was observed in smaller categories of follicles in off fed (Fig. 43B) and high zinc dose groups (Fig. 43CD).

Follicular epithelial layer (theca interna and theca externa) of control group was compactly arranged with clear thecal gland in Group-I (Fig. 44A). Loose arrangement was observed in theca layer (theca interna and theca externa) in all treated groups (Fig. 44BCD) compared to control group. Loose thecal layer, scanty thecal glands and disrupted tissue was observed in off fed group (Fig. 44B) and in both zinc treatment groups (Fig. 44BCD). This was more profound in both off fed (Fig. 44B)

and high dose zinc groups, (30,000ppm zinc/Kg feed) (Fig. 44BCD). The first sign of atresia in primordial and growing follicles was shrinkage. Increased number of atretic follicles were seen in off fed group (Fig. 45B) and birds treated with both zinc doses (Fig. 45CD) whereas in control group, these were not present (Fig. 45A). The cytoplasm of atretic follicles showed thick accumulation of yolky vacuole mass and hypertrophied granulosa cells.

Stromal tissue in treated groups was loosely arranged and more interstitial spaces were seen while in Group-I (control) (Fig. 46A) compact and well organized stroma with less or no lacunae were observed (Fig. 46BCD).

Table 28: Effect of complete restraint feeding and zinc administration on mean body weight (gm) in control and treated groups in White Leghorn layer birds at 67th week of age (N=10)

	Group-I	Group-II	Group-III	Group-IV
Initial Body weight	1493.00±36.17	1526.00±24.92	1478.50±25.36	1514.00±36.31
3rd day	1559.00±24.96	1325.00±41.43	1359.00±28.54	1432.00±49.53
6th day	1583.00±35.50	1209.50±43.48	1294.00±51.41	1342.00±50.94
9th day	1596.00±33.04	1135.00±43.77	1275.00±39.02	1332.50±52.26
12th day	1603.00±28.56^{a*}	1081.50±47.11^{ab****}	1262.00±47.70^{ab****c**}	1323.50±52.14^{ac***b****}

Values are means±SEM (N=10)
a=Initial weight of that group compared to final weight.
b=Group-I compared to all other groups at 12th day
c=Group-II compared to Group-III and group-IV at 12th day.
d=Group-III compared to Group-IV at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 29: Effect of complete restraint feeding and zinc administration on mean body weight (gm) in White Leghorn layer birds at 67th week of age, slaughtered on 12th day of treatment (Batch-I)

	Group-I	Group-II	Group-III	Group-IV
Initial Body weight	1477.00±41.40	1430.00±58.57	1429.00±18.62	1442.00±20.29
3rd day	1498.00±21.54	1230.00±34.50	1290.00±20.82	1384.00±54.82
6th day	1516.00±22.27	1105.00±31.62	1192.00±31.28	1220.00±16.07
9th day	1518.00±27.46	1036.00±36.00	1177.00±57.40	1196.00±35.16
12th day	1550.00±27.20	977.00±50.31^{ab****}	1156.00±56.47^{a***b****}	1191.00±25.76^{ab****c***}

Values are means ± SEM (N=5)
a=Initial weight of that group compared to final weight.
b=Group-I compared to all other groups at 12th day
c=Group-II compared to Group-III and group-IV at 12th day.
d=Group-III compared to Group-IV at 12th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 30: Changes in mean comb and wattle length and width (mm) during restraint feed and zinc administration in White Leghorn (WLH) layer birds at 67th week of age, slaughtered on 12th day of experiment (Batch-I)

Treatment groups	COMB		WATTLES	
	Length	**Width**	**Length**	**Width**
Group-I	98.09±3.01	54.88±1.19	33.79±0.68	33.86±2.67
Group-II	31.17±0.23^{a****}	27.82±0.32^{a****}	25.44±0.26^{a****}	25.83±1.67^{a*}
Group-III	82.77±4.42^{a**b****}	48.17±4.52^{b***}	24.09±2.26^{a***}	29.82±2.41
Group-IV	84.51±1.96^{a***b****}	47.14±2.23^{a**b****}	32.72±1.34^{a*}	26.92±1.19^{a*}

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 31. Effect of complete restrained feeding and zinc administration on mean ovarian, oviduct and liver weight (gm), length and width (mm) in White Leghorn (WLH) layer birds at 67th week of age, slaughtered on 12th day of experiment (Batch-I)

Treatment groups	Ovaries			Oviduct		Liver
	Weight	Length	width	weight	Length	Weight
Group-I	49.86±1.62	47.40±4.43	28.85±3.99	61.25±2.25	73.26±2.30	50.41±4.01
Group-II	5.66±0.60^{a****}	31.15±2.73^{a***}	19.53±3.96	12.81±1.19^{a****}	39.46±2.24^{a****}	19.20±0.63^{a****}
Group-III	11.62±2.00^{a****b*}	33.59±2.10^{a*}	20.79±0.40	20.02±3.59^{a****}	49.89±4.05^{a****b*}	32.43±4.58^{a**b**}
Group-IV	8.94±0.66^{a****b***}	39.42±2.15^{a*}	22.92±1.74	20.11±1.91^{a****b***}	38.60±1.71^{a****}	34.00±3.09^{a***b***}

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 33: Effect of complete restraint feeding and zinc administration on mean plasma estradiol concentration (pg/ml) in White Leghorn (WLH) layer birds at 67th weeks of age, slaughtered on 12th day of experiment (Batch-I)

	Group I	Group-II	Group-III	Group-IV
Initial	214.54±11.95	203.45±13.57	219.02±15.56	215.66±17.25
3rd day	223.06±11.85	93.11±7.41^{a****}	98.12±9.56^{a****}	87.52±11.77^{a****}
6th day	220.70±10.83	67.59±8.38^{a****}	74.57±11.12^{a****}	66.91±9.14^{a****}
9th day	232.97±3.83	42.58±5.19^{a****}	48.32±8.54^{a****}	45.46±7.55^{a****}
12th day	217.90±9.50	29.13±6.68^{a****}	23.41±4.27^{a****}	27.82±3.28^{a****}

Values are means±SEM (N=10)
a= Group-I compared to all other groups.
b= Group-II compared to Group-III and group-IV.
c= Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 34: Effect of complete restraint feeding and zinc administration on mean plasma progesterone concentration (ηg/ml) in White Leghorn (WLH) layer birds at 67th weeks of age, slaughtered on 12th day of experiment (Batch-I)

	Group I	**Group-II**	**Group-III**	**Group-IV**
Initial	3.87±0.06	3.74±0.08	3.64±0.16	3.76±0.07
3rd day	3.94±0.21	1.72±0.06^{a****}	1.77±0.09^{a****}	1.89±0.05^{a****}
6th day	3.72±0.13	1.28±0.08^{a****}	1.22±0.04^{a****}	1.17±0.10^{a****}
9th day	3.68±0.28	0.65±0.22^{a****}	0.81±0.20^{a****}	0.69±0.22^{a****}
12th day	4.26±0.73	0.57±0.18^{a****}	0.84±0.1^{a****}	0.71±0.17^{a****}

Values are means±SEM (N=10)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 35: Effect of complete restraint feeding and zinc administration on mean plasma corticosterone concentration (ηg/ml) in White Leghorn (WLH) layer birds at 67th weeks of age, slaughtered on 12th day of experiment (Batch-I)

	Group I	Group-II	Group-III	Group-IV
Initial	14.24±1.55	15.82±2.01	14.07±2.14	16.01±2.31
3rd day	15.57±3.94	52.97±9.22^{a****}	43.70±7.68^{a****}	40.92±8.06^{a****}
6th day	12.90±5.68	47.71±5.71^{a****}	49.92±8.5^{a****}	45.89±6.07^{a****}
9th day	13.53±4.07	49.10±7.09^{a****}	48.52±10.22^{a****}	44.92±7.23^{a****}
12th day	14.70±2.83	49.15±6.67^{a****}	45.97±9.26^{a****}	47.84±7.98^{a****}

Values are means±SEM (N=10)
a= Group-I compared to all other groups.
b= Group-II compared to Group-III and group-IV.
c= Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 36: Effect of complete restraint feeding and zinc administration on mean number of ovarian yolk follicles in different category range (mm) in White Leghorn layer birds at 67th week of age, slaughtered on 12th day of experiment (Batch-I)

	Yolky Follicles Number Category Range (mm)						
	1-5mm	**5.1-10mm**	**10.1 -15mm**	**15.1-20mm**	**20.1-25mm**	**25.1-30mm**	**>30mm**
Group I	104.80±6.11	9.40±2.66	1.00±0.45	0.80±0.49	1.40±0.51	1.00±0.32	0.80±0.37
Group II	54.60±6.71^{a****}	1.80±0.58^{a****}	(1)	-	-	-	-
Group III	58.00±6.25^{a****}	4.00±1.00	(2)	0.80±0.37	-	-	-
Group IV	42.60±5.64^{a****}	2.80±0.58^{a****}	(1)	-	-	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001
Digits in parenthesis are number of yolky follicle in respective group

Table 37: Effect of complete restraint feeding and zinc administration on mean (mm) diameter category range of ovarian yolky follicles in White Leghorn layer birds at 67th week of age slaughtered on 12th day of molting (Batch-I)

	Yolky Follicles Category Range (mm)						
	1-5mm	**5.1-10mm**	**10.1 -15mm**	**15.1-20mm**	**20.1-25mm**	**25.1-30mm**	**>30mm**
Group I	4.35±0.29	6.14±0.55	11.78±0.53	17.95±0.56	22.96±0.93	28.99±0.53	31.89±0.49
Group II	3.67±0.28^{a**}	6.14±0.32	12.20 (1)	-	-	-	-
Group III	4.14±0.30^{b*}	5.49±0.14	11.58 (2)	16.30±0.48	-	-	-
Group IV	3.76±0.37^{a**}	5.23±0.08^{ab*}	12.67 (1)	-	-	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001
Digits in parenthesis are number of yolky follicle in respective group

Table 38: Effect of complete restraint feeding and zinc administration on mean non yolky follicular diameter (µm) in White Leghorn layer birds at 67th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Follicular Diameter Category Range (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group I	149.82±4.83	305.17±5.51	508.03±6.09	720.52±11.35	872.07±10.62	1415.65±71.45
Group II	132.18±12.12	293.36±12.95	488.42±14.32	674.65±17.33		-
Group III	137.80±3.21[a*]	298.20±6.39	502.61±6.55	706.32±8.66	856.95±10.18	1234.19±43.40
Group IV	131.70±7.26[a*]	295.52±9.78	491.27±10.80	692.73±16.12		-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 39: Effect of complete restraint feeding and zinc administration on mean non yolky oocytes diameter (µm) category range in White Leghorn layer birds at 67th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Oocytes Diameter Category Range (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1000µm**
Group I	111.51±3.84	238.98±5.73	412.33±6.66	617.39±18.03	754.46±9.87	1194.86±67.13
Group II	89.36±9.51[a*]	222.50±10.87	394.36±14.74	563.55±15.62		-
Group III	110.21±2.52[b***]	231.69±5.09	406.42±5.86	604.70±11.49	734.79±11.36	1103.52±49.43
Group IV	103.66±5.27	229.38±7.49	407.41±11.20	580.45±15.31		-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 40: Mean non yolky follicular wall thickness (µm) category range at the end of treatment period in White Leghorn layer birds at 67th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Follicular Wall Thickness Category Range (µm)					
	≤20µm	**21-40µm**	**41-60µm**	**61-80µm**	**81-100µm**	**>101µm**
Group I	15.61±0.11	30.24±0.50	50.22±0.45	66.26±0.90	86.70±0.80	129.23±3.70
Group II	12.33±1.03^{a****}	27.51±1.18^{a*}	44.32±0.92^{a****}	63.67±0.87	-	-
Group III	13.86±0.28^{a****}	29.37±0.58	47.12±0.44^{a****b***}	65.33±0.66	86.26±1.37	139.29±8.78
Group IV	13.62±0.56^{a****}	28.21±1.05	46.46±0.96^{a****}	64.28±1.14	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 41: Effect of complete restraint feeding and zinc administration on mean number of non yolky follicles per section of ovary at the end of treatment period in White Leghorn layer birds at 67th week of age, slaughtered on 12th day of Experiment (Batch-I)

	Mean Follicle Number Category Range (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group-I	28.00±8.37	6.80±0.66	5.00±1.26	2.00±0.32	1.00±0.32	1.40±0.24
Group-II	14.40±2.20^{a**}	4.60±0.51^{a*}	3.20±1.24	-	-	-
Group-III	18.60±4.51	6.20±1.07	3.80±0.86	1.60±0.24	-	-
Group-IV	14.60±1.91^{a**}	5.20±0.58	3.40±0.75	-	-	-

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

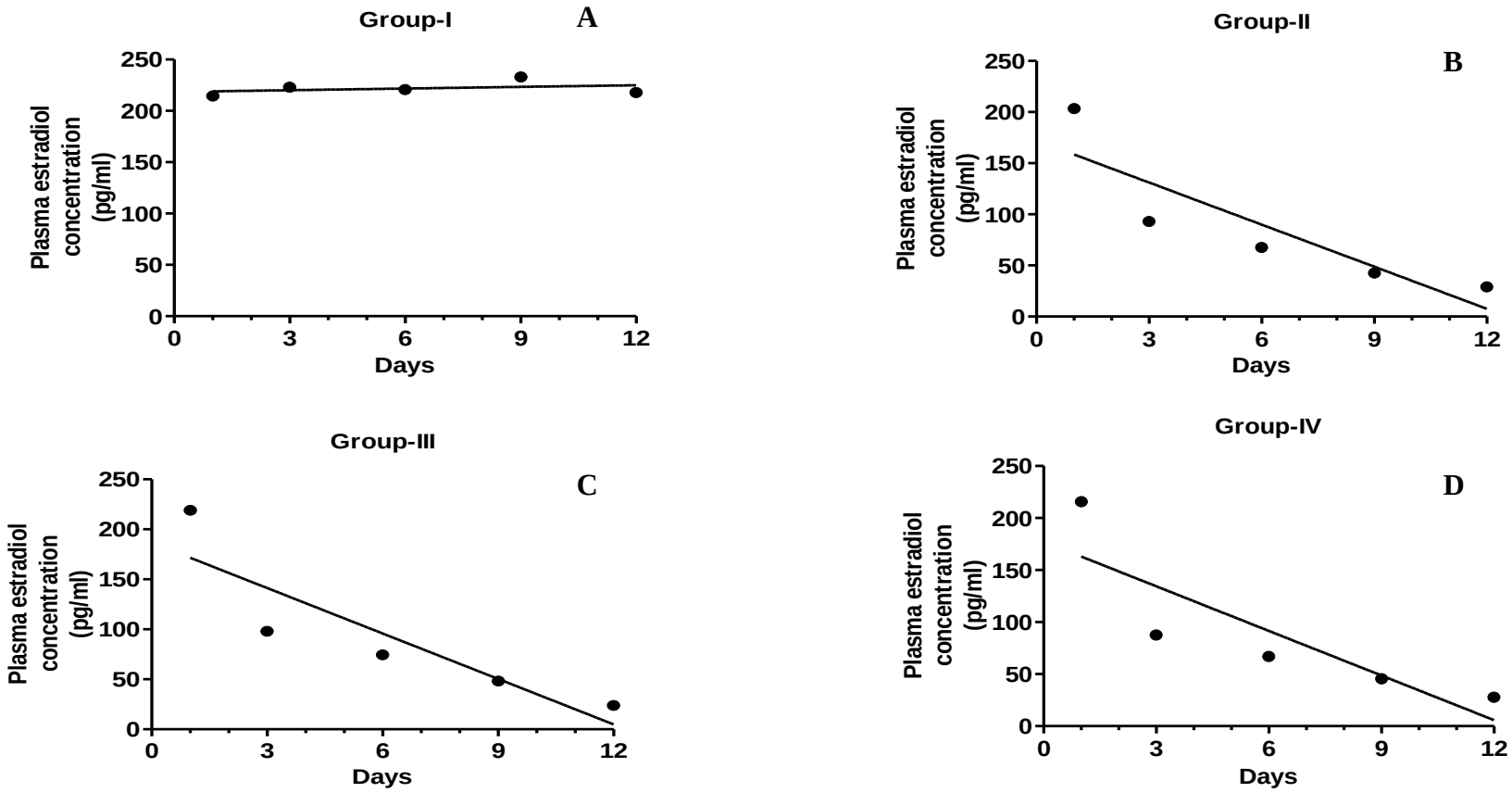


Fig. 35: Regression line showing significant decrease in mean plasma estradiol levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during twelve days of treatment in White Leghorn layer birds at 67th week of age (Batch-I).

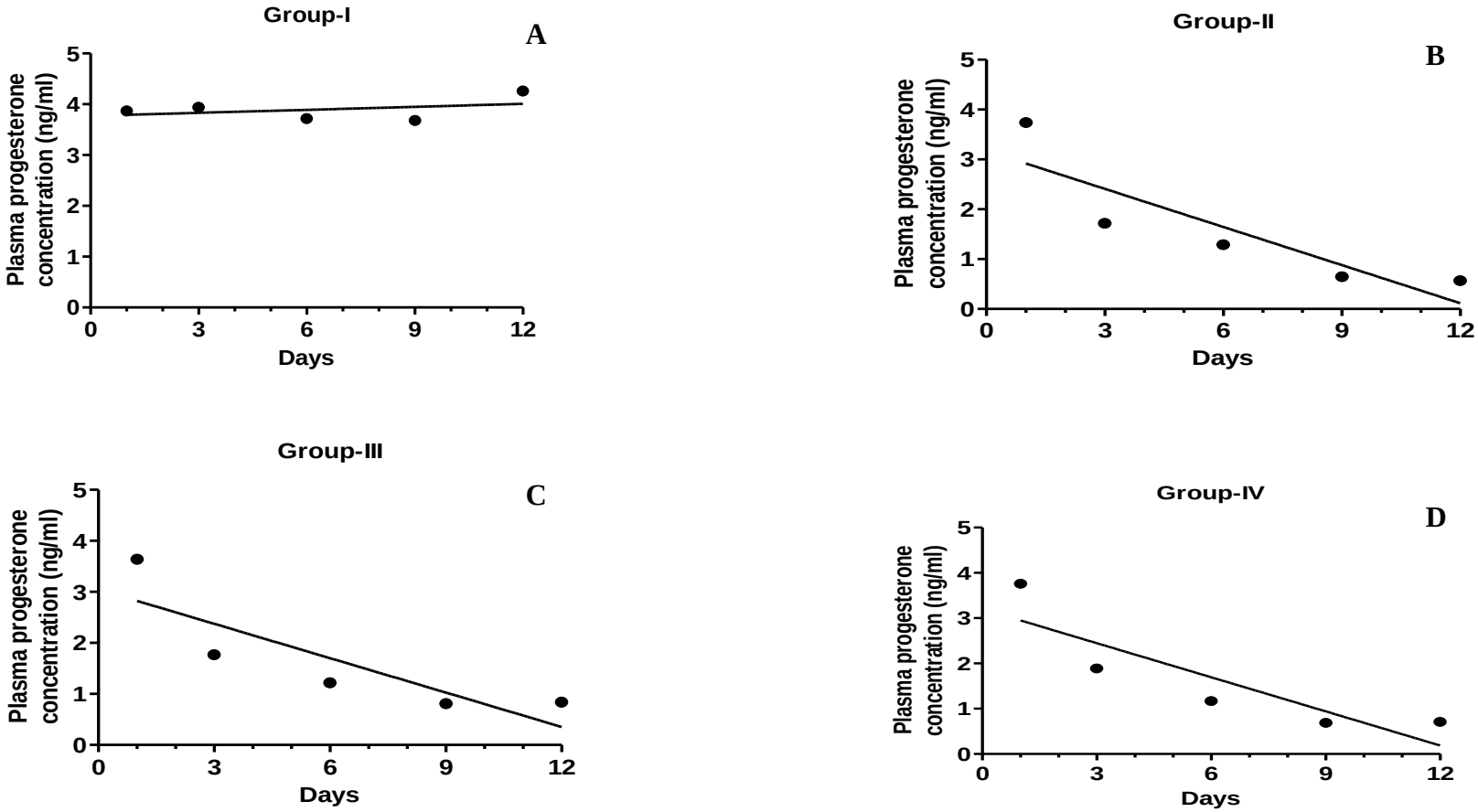


Fig. 36: Regression line showing significant decrease in mean plasma Progesterone levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during twelve days of treatment in White Leghorn layer birds at 67th week of age (Batch-I).

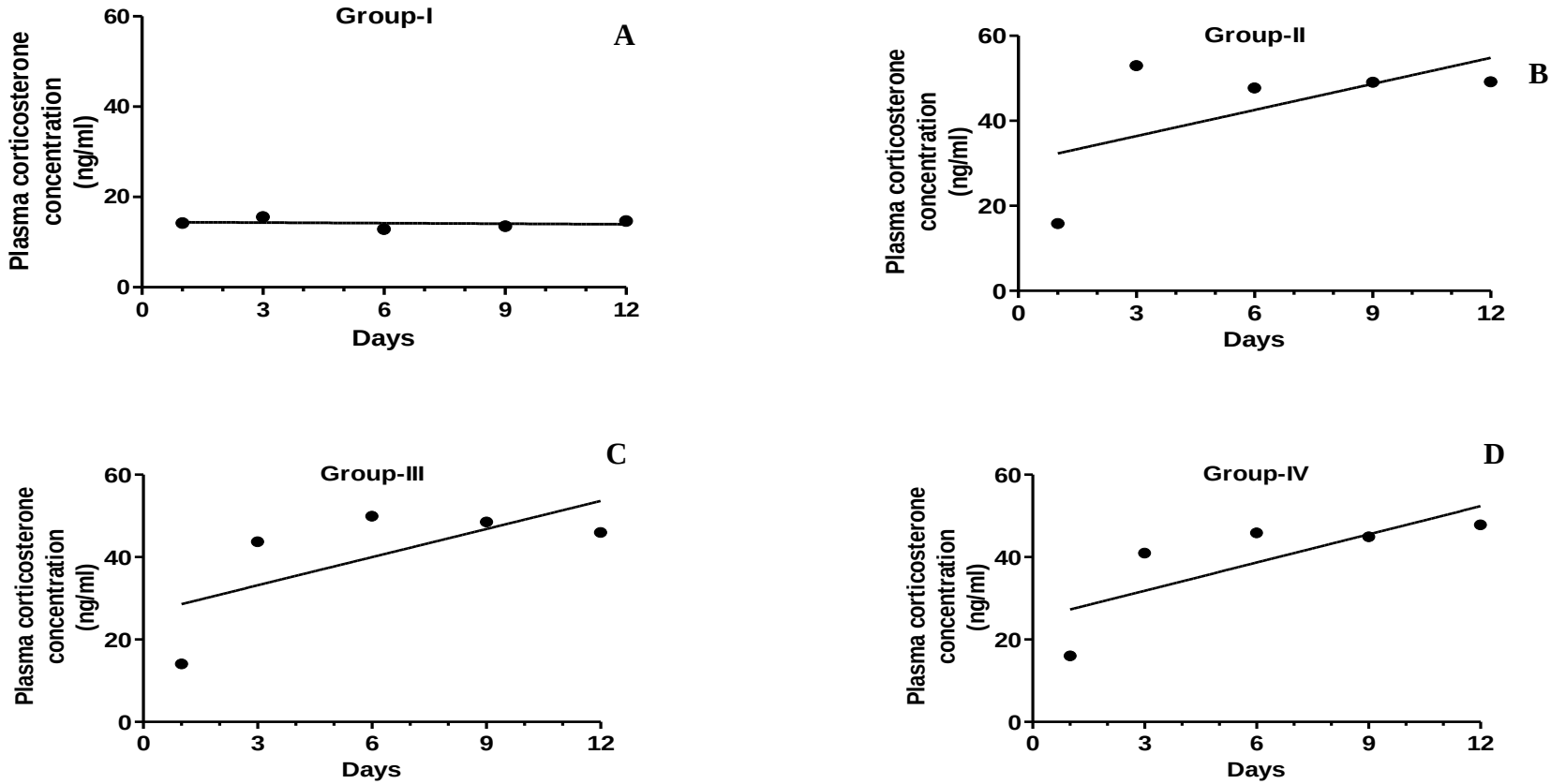


Fig. 37: Regression line showing significant increase in mean plasma corticosterone levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during twelve days of treatment in White Leghorn layer birds at 67th week of age (Batch-I).

BATCH-II

1 BODY WEIGHT

Body weight of birds in each group was measured at an interval of two days and is given in Table 42. On 15th day after withdrawal of molting mean body weight in all treatment groups was also compared with control (Group-I) and among all treatments groups Fig. 47. Mean body weight in each group on 3rd day of withdrawal of molting was compared to final weight of that group at end of the experiment i.e. on 15th day withdrawal of treatment (Fig. 48).

Group-I (Control Group)

The mean body weight of birds on 3rd day after withdrawal of molting was 1631±48.95gm and on 15th day it was 1676±14.35gm. The difference between two was not significant ($t_{(8)}$= 0.88; P=0.40). Regression analysis of variance indicates highly significant increase in mean body weight with the passage of time (b=12.40±2.47; $F_{(1,3)}$=25.18; P=0.02).

Group-II (Off fed)

This group (Batch-II) initially was kept off fed but for next 15 days they were fed normal feed. The mean body weight of birds was 1472.00±44.32gm on 3rd day but after 15-days their mean body weight was 1688.0±44.20gms. There was significant increase in the mean body weight after the withdrawal of treatment ($t_{(8)}$=4.51; P=0.01).

The regression analysis of variance shows that with the passage of time there was highly significant increase in mean body weight due to feeding on normal feed (b=54.80±9.25; $F_{(1,3)}$=35.13; P=0.01).

Group-III (25,000ppm Zinc/Kg Feed)

The remaining five alive birds in this group were slaughtered on 15th day after the withdrawal of treatment (i.e. 15 days after slaughtering of Batch-I). Birds during this period were kept on normal feed and water ad libitum. Their mean body weight was 1528.00±15.94gm on 3rd day after withdrawal of molting but after 15-days their

mean body weight was 1638.0±46.20gms. The increase in mean body weight was significant ($t_{(8)}$=2.25; P=0.05).

Regression analysis of variance showed that due to normal feed and progression of time there was highly significant increase in mean body weight (b=24.80±6.30; $F_{(1,3)}$=15.50; P=0.03).

Group-IV (30,000ppm Zinc/Kg Feed)

The addition of zinc was withdrawn and five birds in this group were fed normal feed. The mean body weight of these was 1533.00±74.36gm on 3rd day after withdrawal of molting and after 15 days mean body weight was 1679.00±70.01gm. The increase in mean body weight was non significant ($t_{(8)}$=1.43; P=0.19) compared to body weight on 3rd day after withdrawal of molting.

Regression analysis of variance also indicated highly significant increase in mean body weight with the passage of time (b=35.50±7.80; $F_{(1,3)}$=20.28; P=0.02).

Comparisons

Comparisons of changes in mean body weight in different groups after the withdrawal of treatment and resumption to normal feed was calculated. On the 15th day of withdrawal treatment mean body weight in Group-II, Group-III and Group-IV was similar to Group-I. The comparison among all treated groups did not show significant difference among each other. During this period of experiment all groups were given normal feed so an increase in mean body weight in all groups was noticed.

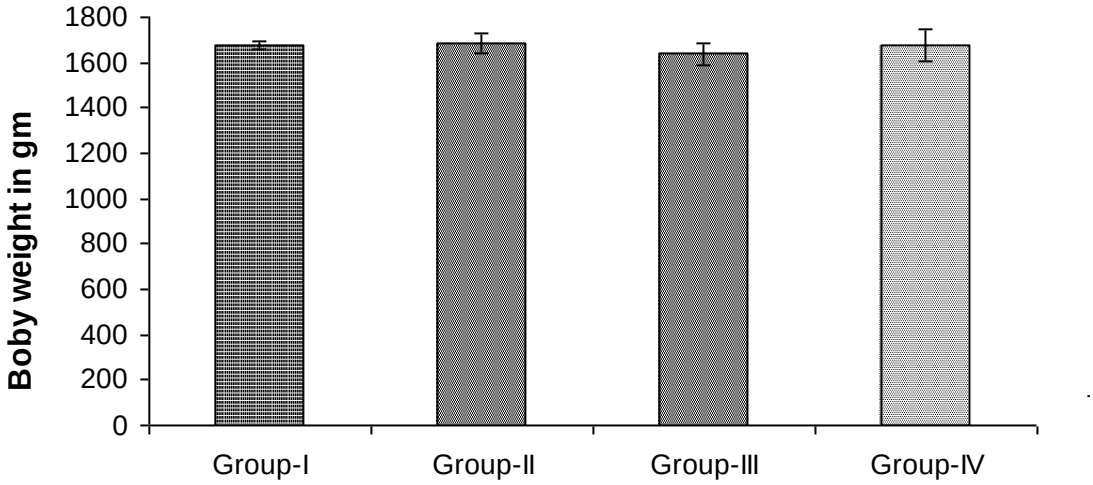


Fig. 47. Changes on mean body weight (gm) during withdrawal of restraint feeding and zinc administration in control and treated groups of White Leghorn (WLH) layer birds at 67th week of age on 15th day of experiment (Batch-II)

b=Group-II compared to Group-III and Group-IV at 15th day
c=Group-III compared to Group-IV at 15th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

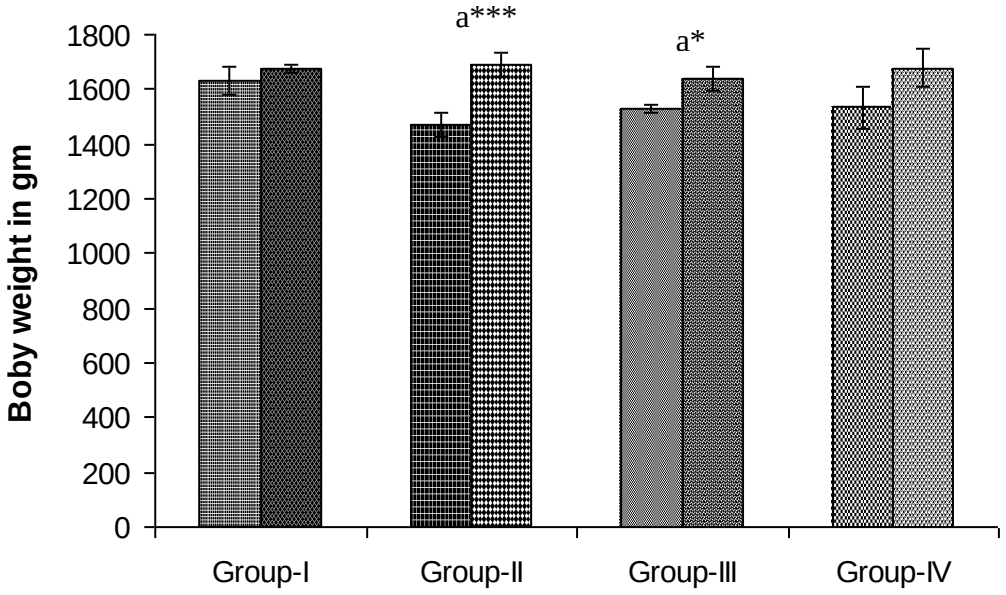


Fig. 48: Comparison of initial body weight and body weight (gm) on 15th day after withdrawal of restraint feeding and zinc administration in control and treated groups at 67th week of age in White Leghorn (WLH) layer birds (Batch-II).

a=Group-I compared to all other groups at 15th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

2 SECONDARY SEXUAL CHARACTERISTICS

Comb and Wattles

Mean length and width of comb and wattles in all groups is represented in Table 43.

Comb

After the withdrawal of treatment and resumption to normal feed significantly lower comb length was observed in Group-II ($t_{(8)}$=5.15; P=0.001) and Group-IV ($t_{(8)}$=2.33; P=0.05) but in Group-III ($t_{(8)}$=1.00; P=0.35) there was no significant difference compared to the Group-I (control group). In comparison of Group-II (off fed) vs zinc treated Group-III and Group-IV, a significant greater length was observed in Group-III ($t_{(8)}$=4.03; P=0.004) but no significant increase in Group-IV ($t_{(8)}$=1.95; P=0.09) was observed. Mean comb length comparison of Group-III vs Group-IV ($t_{(8)}$=1.50; P=0.17) showed no significant difference.

Similarly, after the withdrawal of treatment and resumption to normal feed significant low comb width was still observed in Group-II ($t_{(8)}$=4.21; P=0.003) compared to Group-I (control). The width in zinc treated groups increased to the level of control group with the result that no significant change was noticed in Group-III ($t_{(8)}$=0.04; P=0.97) and Group-IV ($t_{(8)}$=0.59; P=0.57) which was similar to Group-I. Group-III ($t_{(8)}$=3.64; P=0.01) and Group-IV ($t_{(8)}$=3.80; P=0.01) showed highly significant increase in comb width compared to Group-II.

Wattles

Mean wattles length in Group-II was significantly lower than control group ($t_{(8)}$=2.90; P=0.02), but no significant difference in wattles length of Group III and Group-IV was observed compared to Group-I (control). There was significant increase in wattles length in Group-IV ($t_{(8)}$=2.75; P=0.02) and non significant increase in wattles length in Group-III ($t_{(8)}$=0.80; P=0.45) compared to Group-II (off fed). Group-III vs Group-IV showed no significant difference in wattles length ($t_{(8)}$=0. 96; P=0.37).

Wattles width was significantly lower in Group-II ($t_{(8)}$=17.05; P<0.0001) but difference in Group-III ($t_{(8)}$=1.16; P=0.28) and Group-IV ($t_{(8)}$=0.66; P=0.53) was not significant compared Group-I. Group-III ($t_{(8)}$=2.71; P=0.03) and Group-IV ($t_{(8)}$=5.05; P=0.001) showed significant increase compared to Group-II. Wattles width in Group-III vs Group-IV ($t_{(8)}$=0.10; P=0.92) was not significantly different.

Table 43: Changes in mean comb and wattles length and width (mm) on 15th day after withdrawal of restraint feed and zinc administration in WLH layer birds slaughtered at 67th week of age (Batch-II)

Treatment groups	COMB		WATTLES	
	Length	**Width**	**Length**	**Width**
Group-I	103.31±2.33	56.27±2.27	35.16±2.01	34.34±0.46
Group-II	81.49±3.54^{a****}	40.79±2.89^{a***}	27.86±1.51^{a**}	25.57±0.22^{a****}
Group-III	99.66±2.80^{b*}	56.11±3.06^{b***}	30.70±3.22	31.68±2.25^{b*}
Group-IV	92.14±4.18^{a*}	54.44±2.13^{b***}	34.21±1.74^{b**}	33.30±1.52^{b****}

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

3. EFFECT ON OVARY, OVIDUCT AND LIVER

Ovarian weight, length and width, oviductal weight and width and liver weight after withdrawal of treatment and resumption to normal feed for 15 days are presented in Table 44.

In this Batch-II mean ovarian weight in all treated groups increased and became almost equal to weight in Group-I (control). Comparisons between groups showed non significant difference in Group-I vs Group-II, Group-III and Group-IV ($t_{(8)}$=0.29; P=0.78; $t_{(8)}$=0.02; P=0.99; $t_{(8)}$=0.28; P=0.78) respectively. Group-II compared to Group-III and Group-IV ($t_{(8)}$=0.31; P=0.77; $t_{(8)}$=0.45; P=0.66) also showed no significant change in ovarian weight. Group-III vs Group-IV comparison did not differ significantly ($t_{(8)}$=0.28; P=0.79).

The mean ovarian length in Group-II and Group-IV was significantly lower ($t_{(8)}$=7.17; P<0.0001; $t_{(8)}$=3.21; P=0.01) but in Group-III ovarian length was no significantly ($t_{(8)}$=1.10; P=0.30) different than Group-I although treatment was withdrawal. Ovarian length in Group-II was significantly less than Group-III ($t_{(8)}$=5.38; P=0.001), but non-significantly lower in Group-II compared to Group-IV ($t_{(8)}$=2.05; P=0.08). In Group-III vs Group-IV ($t_{(8)}$=2.18; P=0.06) difference was also non significant.

In Batch-II, mean ovarian width was significantly less in Group-II and Group-IV ($t_{(8)}$=2.99; P=0.02; $t_{(8)}$=3.32; P=0.01) but in Group-III ($t_{(8)}$= 1.27; P=0.24) it was non significantly less compared to Group-I (control). The comparison between Group-II vs Group-III and Group-IV ($t_{(8)}$=2.04; P=0.08; $t_{(8)}$=0.13; P=0.90) showed non significant differences. In Group-III vs Group-IV ($t_{(8)}$=2.44; P=0.04) comparison significantly lower ovarian width was observed in Group-IV.

Mean oviductal weight was still significantly lower in Group-II ($t_{(8)}$=2.10; P=0.02) but in Group-III and Group-IV ($t_{(8)}$=0.34; P=0.74; $t_{(8)}$=1.83; P=0.11) no significant difference compared to Group-I (control) was observed indicating that it was improved after the withdrawal of treatment. The comparison of Group-II vs Group-III ($t_{(8)}$=3.93; P=0.01) showed significant increase in Group-III but no significant change in Group-IV was observed ($t_{(8)}$=1.35; P=0.21) compared to Group-II. In Group-III vs Group-IV ($t_{(8)}$=2.26; P=0.05) a significant increase was observed in Group-III.

Mean length of oviduct was significantly smaller in Group-II (off fed) ($t_{(8)}$=4.58; P=0.002) and Group-IV ($t_{(8)}$=2.99; P=0.02) but reduction in Group-III ($t_{(8)}$=1.55;

P=0.16) was not significant compared to Group-I (control). No significant difference was noticed in mean length of oviduct in comparisons of Group-II vs Group-III and Group-IV ($t_{(8)}$=1.61; P=0.15; $t_{(8)}$=0.46; P=0.66) and Group-III vs Group-IV ($t_{(8)}$=1.02; P=0.34).

Reversion to normal feed has significantly increased mean liver weight in all birds and became equal to Group-I (control). Mean liver weight in all groups did not show significant difference when compared with each other.

4. ZINC DEPOSITION IN DIFFERENT ORGANS TISSUE

Deposition of zinc in ovary, Kidney and Liver (µg/gm) in Group-I, Group-II, Group-III (25,000ppm zinc/kg feed) and Group-IV (30,000ppm zinc/kg feed) is given in Table 45.

Deposition of Zinc in Ovary

In Batch-II, there was no appreciable difference in zinc deposition in the ovary of Group-II ($t_{(8)}$=1.56; P=0.16) birds and Group-III (25,000ppm zinc/kg feed) ($t_{(8)}$=1.12; P=0.29) however, birds in Group-IV (30,000ppm zinc/kg feed) ($t_{(8)}$=4.99; P=0.001) showed significantly higher zinc deposition compared to Group-I (control) even after withdrawal of treatment. Within treatment groups, Group-IV ($t_{(8)}$=5.62; P=0.001) showed significant higher zinc deposition compared to Group-III and Group-II.

Deposition of Zinc in Liver

In this experiment, in Batch-II the birds slaughtered after 15-days of withdrawal of treatment zinc deposition in the liver of Group-III (25,000ppm zinc/kg feed) ($t_{(8)}$=5.04; P=0.001) as well as Group-IV (30,000ppm zinc/kg feed) ($t_{(8)}$=10.25; P<0.0001) was significantly higher compared to Group-I (control) even after the withdrawal of zinc treatment. There was highly significant increased zinc deposition in Group-III (25,000ppm zinc/kg feed) ($t_{(8)}$=4.93; P=0.001) and Group-IV (30,000ppm zinc/kg feed) ($t_{(8)}$=10.20; P<0.001) compared to Group-II (off fed). Liver of high zinc dose treated Group-IV also showed significantly ($t_{(8)}$=5.23; P=0.001) greater deposition of zinc compared to Group-III.

Deposition Of Zinc In Kidneys

After the withdrawal of treatment and resumption to normal feed in Batch-II, there was no significant difference in zinc deposition in the kidney of Group-I and Group-II ($t_{(8)}$=0.61; P=0.56) and birds treated with 25,000ppm zinc/Kg feed (Group-III) ($t_{(8)}$=1.89; P=0.09), however, birds treated with 30,000ppm zinc/Kg feed ($t_{(8)}$=10.13; P<0.0001) showed significantly higher zinc deposition compared to Group-I (control). There was also significantly higher zinc deposition in Group-III ($t_{(8)}$=2.57; P=0.03) and Group-IV ($t_{(8)}$=11.65; P<0.0001) compared to Group-II (off fed). In Group-III vs Group-IV zinc deposition was significantly higher ($t_{(8)}$=7.32; P<0.0001) in Group-IV.

Table 45: Mean Zinc deposition concentration (µg/g) in ovary, kidney and liver in WLH layer birds at 67th week of age, slaughtered on 15th day after withdrawal of restraint feed and zinc administration. (Batch-II)

Treatment groups	Batch-II (slaughtered on 15th day after withdrawal treatment)		
	Ovary	**Liver**	**Kidney**
Group-I	7.96±0.42	6.51±0.46	15.31±1.65
Group-II	5.91±0.47	1.57±0.35	13.56±1.22
Group-III	8.22±0.31	$24.96\pm2.34^{ab****}$	$18.79\pm1.62^{b*}$
Group-IV	$15.73\pm1.08^{abc****}$	$45.25\pm2.50^{abc****}$	$33.64\pm1.22^{abc****}$

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

5 HORMONAL ESTIMATION

After withdrawal of treatments and resumption to normal feed in Batch-II mean plasma concentration of Estradiol, Progesterone and Corticosterone are shown in Tables 46, 47 and 48 respectively.

Plasma Estradiol Level

The plasma concentration of estradiol in the different treatment groups is shown in Table 46. The plasma estradiol in each group was recorded after every two day interval up to day 15 (end of experiment). It was found that after 3rd day the estradiol level started increasing in all the treatment groups up to 15th day of the withdrawal of treatment but remained low throughout the experimental period compared to control. On day 3 after withdrawal of treatment and resumption to normal feed the plasma estradiol level in Group-II ($t_{(8)}$=8.47; P<0.0001), Group-III ($t_{(8)}$=8.27; P <0.0001) and Group-IV ($t_{(8)}$=9.04; P <0.0001) was significantly low compared to Group-I (control). Similarly, on 6th day the plasma estradiol level in Group-II ($t_{(8)}$=10.20; P<0.0001), Group-III ($t_{(8)}$=10.01; P<0.0001) and Group-IV ($t_{(8)}$= 10.02; P<0.0001) was significantly lower than Group-I (control). This trend of significant low plasma estradiol level continued in all the treatments on 9th day in Group-II ($t_{(8)}$=5.35; P=0.0007), Group-III ($t_{(8)}$=5.78; P=0.0004) and Group-IV ($t_{(8)}$=5.74; P=0.0004). On 12th day the plasma estradiol levels in Group-II ($t_{(8)}$=4.36; P=0.002), Group-III ($t_{(8)}$=4.63; P=0.002) and Group-IV ($t_{(8)}$=5.39; P=0.0007) was significantly lower compared to Group-I (control). On 15th day plasma estradiol level was non-significantly lower in all treatment groups than Group-I. There was no significant differences in the estradiol level among different treatment groups when they compared to each other on days 3rd, 6th, 9th, 12th and 15th day.

Linear regression analysis of variance showed that there was no significant difference in plasma estradiol concentration with the advance in days of treatment withdrawal (b=0.02±1.37; $F_{(1,3)}$=0.0002; P=0.99) in control (Group-I), whereas there was highly significant increase in plasma estradiol concentration in Group-II (off fed) (b=26.25±6.13; $F_{(1,3)}$=18.34; P=0.02), Group-III (25,000ppm/Kg) (b=26.73±6.14; $F_{(1,3)}$=18.97; P=0.02) and Group-IV (30,000ppm/Kg) (b=28.68±5.70; $F_{(1,3)}$=25.32; P=0.02) with the advance in the days after withdrawal of treatments (Fig. 49).

Plasma Progesterone Level

The plasma concentration of progesterone in the different treatments is shown in Table 47. On 3rd day after withdrawal of treatment and resumption to normal feed mean plasma progesterone level in Group-II ($t_{(8)}$=3.29; P=0.01), Group-III ($t_{(8)}$=2.73; P=0.03) and Group-IV ($t_{(8)}$=3.13; P=0.01) was significantly low compared to Group-I (control). On 6th day highly significant decrease in mean plasma progesterone level was observed in all the treatment groups i.e. Group-II ($t_{(8)}$=3.46; P=0.009), Group-III ($t_{(8)}$=2.62; P=0.03) and Group-IV ($t_{(8)}$=3.08; P=0.02) compared to Group-I (control) after withdrawal of treatment. On 9th day after withdrawal of treatment plasma level of progesterone remained lower in Group-II ($t_{(8)}$=5.27; P=0.0008), Group-III ($t_{(8)}$=4.51; P=0.002) and Group-IV ($t_{(8)}$=5.89; P=0.0004) compared to Group-I (control). Comparison of groups on 12th day revealed that plasma progesterone concentration significantly low in Group-II ($t_{(8)}$=2.52; P=0.04), Group-III ($t_{(8)}$=2.97;P=0.02) and Group-IV ($t_{(8)}$=2.34; P=0.05) compared to Group-I (control). Mean progesterone profile in all treated groups continued to be lower in Group-II ($t_{(8)}$=4.03; P=0.004), Group-III ($t_{(8)}$=3.05; P=0.02) and Group-IV ($t_{(8)}$=3.47; P=0.008) on 15th day after withdrawal of treatment compared to Group-I (control). Comparison among all treatment groups showed no significant difference in mean plasma progesterone level on 3rd, 6th, 9th, 12th and 15th day of experiment but steady increase in progesterone level was observed from 3rd to 15th day after withdrawal of treatment.

Linear regression analysis of variance showed that there was significant increase in plasma progesterone concentration with the advance in days of treatment withdrawal (b=0.13±0.03; $F_{(1,3)}$=16.79; P=0.03) in control (Group-I), Group-II (off fed) (b=0.40±0.07; $F_{(1,3)}$=31.10; P=0.01), Group-III (25,000ppm/Kg) (b=0.37±0.09; $F_{(1,3)}$=17.77; P=0.02) and Group-IV (30,000ppm/Kg) (b=0.35±0.08; $F_{(1,3)}$=20.42; P=0.02) (Fig. 50).

Plasma Corticosterone Level

The plasma concentration of corticosterone in the different treatments is shown in Table 48. Mean corticosterone level remained significantly elevated in Group-II ($t_{(8)}$=3.66; P=0.007), Group-III ($t_{(8)}$=3.22; P=0.01) and Group-IV ($t_{(8)}$=3.52; P=0.008) compared to Group-I (control) on 3rd day after withdrawal of treatment. Analysis of corticosterone level on 6th day after withdrawal of treatment in chicks revealed significant higher levels in Group-II ($t_{(18)}$=3.89; P=0.005), Group-III ($t_{(8)}$=4.23; P=0.003) and Group-IV ($t_{(8)}$=3.46; P=0.009) compared to Group-I (control). Mean corticosterone concentration on 9th day after withdrawal of treatment in Group-II ($t_{(8)}$=3.70; P=0.006), Group-III ($t_{(8)}$=3.94; P=0.004) and Group-IV ($t_{(8)}$=5.84; P=0.0004) showed highly significant rise compared to Group-I (control). On 12th day significantly higher level of corticosterone in plasma were also observed in Group-II ($t_{(8)}$=4.24; P=0.003), Group-III ($t_{(8)}$=3.10; P=0.01) and Group-IV ($t_{(8)}$=4.53; P=0.002) compared to Group-I (control) after withdrawal of treatment. On day fifteen (15) after withdrawal of treatment plasma corticosterone levels remained higher in treatment Group-II ($t_{(8)}$=2.60; P=0.03), Group-III ($t_{(8)}$=2.50; P=0.04) and Group-IV ($t_{(8)}$=2.82; P=0.02) compared to Group-I (control). Comparison among all treatment groups showed no significant difference in mean plasma corticosterone concentration throughout the period after withdrawal of treatment.

Linear regression analysis of variance shows that there was no significant difference in plasma corticosterone concentration with the advance in days of treatment withdrawal (b=-0.27±0.27; $F_{(1,3)}$=0.99; P=0.39) in control (Group-I), whereas there was highly significant decrease in plasma corticosterone concentration in Group-II (b=-2.37±0.69; $F_{(1,3)}$=11.48; P=0.04), Group-III (25,000ppm/Kg) (b=-1.44±1.08; $F_{(1,3)}$=1.78; P=0.27) and Group-IV (30,000ppm/Kg) (b=-1.99±0.59; $F_{(1,3)}$=11.08; P=0.04) with the advance in the days after withdrawal of treatment (Fig. 51).

6 MORPHOMETRY

Mean Ovarian Yolky Follicles Numbers

In Batch-II chicks were fed normal feed in all treatment groups and slaughtered after 15 days of withdrawal of treatment. Number of yolky follicle was recorded in control and all the treatment groups which is shown in Table 49. In category 1-5mm mean number of follicles had decreased in all groups however, in Group-II mean follicles number was significantly low ($P<0.05$) compared to Group-I. One way analysis of variance also showed highly significant difference in means among different groups ($F_{(3,16)}=3.50$; $P=0.04$). In category 5.1-10mm no significant difference was observed among all treatment groups.

In all other categories of follicles there is no significant difference in mean yolky numbers in all treatment groups compared to control group. The results indicated that reversion to normal feed has improved the growth of yolky follicles in all the categories of follicles.

Mean Ovarian Yolky Follicles Diameter

In Batch-II mean diameter of yolky follicles were arranged in different category ranges according to their size are given in table 50. One way analysis of variance showed that there was no significant difference in mean diameter of yolky follicles in all categories compared to control.

In categories from 10.1-15mm–30.1-35mm either there was no representation of yolky follicles or number of yolky follicle was so low that it was not advisable to carry out comparisons in their categories.

Mean Non Yolky Ovarian Follicular Diameter

Mean diameter of non yolky ovarian follicles at the end of treatment period is given in Table 51. Ovarian follicles with lowest diameter to highest diameter were categorized with a class interval of 200µm. In this batch follicular diameter category range from ≤200µm to >1001µm did not show significant difference compared to Group-I. There was also no significant difference observed on comparison among all treatments groups. This indicates reversion of birds to normal feed has improved the follicular growth.

Mean Non Yolky Oocyte Diameter

In Batch-II of experiment-II treatment in all the groups was withdrawn to see if the effect of treatment persists after the withdrawal of treatment. Oocyte diameter (µm) in all groups is arranged in categories presented in Table 52. In category ≤200µm of Group-II there was significant decrease ($t_{(133)}$=2.54; P=0.01) in mean oocyte diameter compared to Group-I. Similarly, Group-II compared to Group-III showed significant decrease in oocyte diameter in same category ($t_{(134)}$=2.03; P=0.04). There was highly significant decrease in mean oocyte diameter in category 601-800µm of Group-II, Group-III and Group-IV ($t_{(30)}$=6.80; P<0.0001; $t_{(28)}$=5.11; P<0.0001; $t_{(40)}$=7.75; P<0.0001) compared to Group-I. In categories 201-400µm, 401-600µm, 801-1000µm and >1001µm mean oocyte diameter in all treated groups did not differ significantly compared to Group-I (control).

Mean Non Yolky Follicular Wall Thickness

Mean non yolk follicular wall thickness (μm) arranged in different categories in four groups is given in Table 53. The mean follicular wall thickness in the category ≤20μm in Group-II, Group-III and Group-IV ($t_{(138)}$=6.14; P<0.0001; $t_{(123)}$=5.74; P<0.0001; $t_{(178)}$=7.12; P<0.0001 respectively) was significantly lesser compared to Group-I after the withdrawal of treatment. Also in the category 21-40μm mean follicular thickness was significantly low in Group-II, Group-III and Group-IV ($t_{(152)}$=8.55; P<0.0001; $t_{(165)}$=5.46; P<0.0001; $t_{(208)}$=6.16; P<0.0001 respectively) compared to Group-I. Mean follicular wall thickness of categories 41-60μm and 61-80μm in all treatment groups were similar to the control group.

Group-II and Group-IV ($t_{(30)}$=3.47; P=0.002; $t_{(40)}$=2.24; P=0.0003) showed significant decrease in follicular wall thickness compared to Group-I in category 81-100μm and Group-II also has significant reduction in follicular wall thickness in this category compared to Group-III ($t_{(20)}$=2.27; P=0.03). Group-II compared to Group-I and zinc treated groups showed significant decrease in follicular wall thickness in category >101μm.

Mean Non Yolky Ovarian Follicle Number

Mean non yolk ovarian follicles number in different categories in relation to follicle diameter has been arranged in all groups after withdrawal of treatment and resumption to normal feed which is presented in Table 54. In this batch follicular number in all categories range from ≤200μm to >1001μm did not show significant difference compared to Group-I (control). As in Batch-II of experiment-I, follicles developed in the follicular categories 601-800 μm, 801-1000μm and >1001μm in all groups. There was also no significant difference in follicular number in comparisons among treatment groups as well. This indicates reversion of birds to normal feed has improved the follicular growth and numbers.

7 HISTOMORPHOLOGY

General and Histological Observations of Ovaries

In this batch treatment was withdrawn and birds were kept for fifteen (15) days on normal feed before slaughtering. Ovarian weight and size in off fed, low and high zinc dose treatment groups increased during this period and was comparable to Group-I (Table 44). After slaughtering the birds their ovaries were removed carefully. Large yellow yolky and small whitish (transparent) yolky follicles were counted and measured (Table 49 and 50). In ovaries of control and treated birds yellow yolky and small whitish yolky follicles were observed however, yellow yolky follicles were less in all birds. Blood vessels were visible in yolky follicles. Microscopic study of cross section of middle portion of ovary of the White Leghorn layer birds at 67^{th} week of age showed that it possessed two distinct regions, an outer cortex and inner medulla. The cortex contained follicles of variable sizes range. The inner medullar stroma was composed of well vascularised and innervated connective tissue with lacunae and blood vessels.

The control ovary contained number of healthy small and large follicles of diameter category ranging from ≤200 to >1001µm at various developmental stages. Number of ovarian follicles in Group-II, Group-III and Group-IV (Fig. 52BCD) of all categories also increased compared to control group (Fig. 52A). Follicles of larger categories were present in both zinc treated groups and off fed group after the withdrawal of treatment (Table 51).

Oocytes of control group possessed well defined spherical nucleus with distinct network of chromatin and clear nuclear membrane (Fig. 53A) and all treatment groups also have similar appearance (Fig. 53BCD). Normal feeding of the birds after withdrawal of treatment improves the structure of nucleus and nuclear matrix.

There was no difference in structure of primordial follicles in treated groups compared to control (Fig. 54A). In this batch treated bird ovary contained normal ooplasm in follicles (Fig. 54BCD). These oocytes were attached to the granulosa layer with cytoplasmic extension. Tiny cytoplasmic extension from the granulosa passed through the zona pellucid connecting them to the oocyte.

The thin layer of zona pellucida between ooplasm and granulosa layer of follicle with well defined cytoplasmic processes were seen in control (Fig. 55A) and all treated groups (Fig. 55BCD) after the withdrawal of treatment and resumption to the normal feed.

In larger follicle oocytes were surrounded by a single layer of granulosa cells with strong basal membrane. Theca was distinguishable into two layers, i.e. theca interna and theca externa in all groups after the withdrawal of treatment. Basal lamina layer was normal and no disruption in it structure was observed in control (Fig. 56A) and all treated groups. Little reduction in thickness of peripheral follicular epithelium (theca interna and theca externa) and granulosa layers was seen in all categories of follicles in off fed (Fig. 56B) and low and high dose zinc (Fig. 56CD) compared to control.

Follicular epithelium layer (theca interna and theca externa) in follicles of control group (Fig. 57A) and all treated birds was compactly arranged with clear thecal gland (Fig. 57BCD) in the theca interna. However, the concentration of thecal gland in control group (Fig. 58A) was higher compared to all treatment groups (Fig. 58BCD).

The normal feeding also improved overall condition of ovarian follicles. Scanty atretic follicles were seen in off fed group and birds treated with zinc. No atretic primordial follicles were observed in treated groups due to normal feeding of treated birds and interstitial spaces were also seen less (Fig. 59A). Compact and well organized stromal tissue was seen with smooth connective tissue (Fig. 59BCD).

Table 42: Changes in mean body weight (gm) in White Leghorn (WLH) layer birds at 67th week of age slaughtered on 15th day after the withdrawal of restraint feeding and zinc administration (Batch-II)

Days after moulting	Group-I	Group-II	Group-III	Group-IV
3rd day	1631.00±48.95	1472.00±44.32	1528.00±15.94	1533.00±74.36
6th day	1636.00±45.45	1572.00±42.71	1594.00±34.87	1611.00±60.09
9th day	1666.00±43.08	1598.00±52.29	1614.00±31.72	1648.00±72.00
12th day	1670.00±35.78	1688.00±48.00	1622.00±27.82	1674.00±79.54
15th day	1676.00±14.35	1688.00±44.20^{a***}	1638.00±46.20^{a*}	1679.00±70.01

Values are means ± SEM (N=5)
a=Initial weight of that group compared to final weight.
b=Group-I compared to all other groups at 15th day
c=Group-II compared to Group-III and Group-IV at 15th day
d=Group-III compared to Group-IV at 15th day
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 44: Mean ovarian, oviduct and liver weight (gm), length and width (mm) in WLH layer birds at 67th week of age slaughtered on 15th day after withdrawal of restraint feed and zinc administration (Batch-II)

Treatment groups	Ovaries			Oviduct		Liver
	Weight	Length	width	weight	Length	Weight
Group I	59.13±2.63	64.01±2.36	36.69±3.94	69.46±3.17	82.20±2.65	52.97±4.46
Group II	57.42±5.26	41.30±2.12^{a****}	23.06±2.31^{a**}	58.74±1.66^{a**}	68.20±1.52^{a***}	52.92±3.99
Group III	59.19±2.37	60.02±2.76^{b****}	30.51±2.83	70.95±3.08^{b***}	74.90±3.88	51.23±1.85
Group IV	60.88±5.52	49.99±3.68^{a***}	23.39±0.74^{a***c*}	62.44±2.18^{c*}	69.80±3.18^{a**}	52.95±5.64

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 46: Mean plasma estradiol concentration (pg/ml) in White Leghorn (WLH) layer birds at 67th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Group-I	Group-II	Group-III	Group-IV
3rd day	221.19±18.55	50.01±8.01^{a****}	57.93±6.74^{a****}	43.72±6.44^{a****}
6th day	228.15±14.46	59.33±8.05^{a****}	69.38±6.51^{a****}	58.41±8.84^{a****}
9th day	225.56±26.23	61.90±15.76^{a****}	72.75±3.46^{a****}	66.21±9.11^{a****}
12th day	218.96±17.41	111.13±17.61^{a***}	117.36±13.37^{a***}	109.08±10.62^{a****}
15th day	225.88±68.65	155.36±14.34	167.59±11.42	161.80±10.19

Values are means±SEM (N=10)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 47: Mean plasma progesterone concentration (ηg/ml) in White Leghorn (WLH) layer birds at 67th weeks of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Group I	Group-II	Group-III	Group-IV
3rd day	4.14±0.76	1.29±0.42^{a***}	1.77±0.43^{a*}	1.61±0.29^{a***}
6th day	4.20±0.66	1.68±0.30^{a***}	1.94±0.55^{a*}	1.70±0.46^{a**}
9th day	4.44±0.38	1.72±0.35^{a****}	1.91±0.42^{a***}	1.79±0.25^{a****}
12th day	4.37±0.47	2.65±0.50^{a*}	2.76±0.28^{a**}	2.62±0.59^{a*}
15th day	4.71±0.24	2.79±0.41^{a***}	3.20±0.43^{a**}	2.88±0.47^{a***}

Values are means±SEM (N=10)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 48: Mean plasma corticosterone concentration (ηg/ml) in White Leghorn (WLH) layer birds at 67th weeks of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Group I	Group-II	Group-III	Group-IV
3rd day	14.55 ±2.43	37.11±5.68^{a***}	33.14 ±5.24^{a***}	39.55 ±6.67^{a***}
6th day	13.77±2.26	34.49±4.82^{a***}	34.42 ±4.33^{a***}	33.51 ±5.24^{a***}
9th day	12.46±1.19	36.47±6.39^{a***}	34.64 ±5.50^{a***}	35.31 ±3.72^{a****}
12th day	14.17±2.39	31.00±3.17^{a***}	34.45 ±6.08^{a***}	32.83 ±3.36^{a***}
15th day	13.01±3.83	27.03±3.79^{a*}	25.92±3.48^{a*}	29.92±4.60^{a**}

Values are means±SEM (N=10)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 49: Mean number category range (mm) of ovarian yolky follicles in White Leghorn layer birds at 67th weeks of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	1-5mm	5.1-10mm	10.1 -15mm	15.1-20mm	20.1-25mm	25.1-30mm	30.1-35 mm
Group I	103.40±3.80	5.80±1.66	1.20±0.49	1.40±0.51	1.80±0.58	1.80±0.66	1.40±0.68
Group II	83.00±4.60[a*]	3.00±0.71	0.80±0.37	(1)	(2)	(1)	1.00±0.32
Group III	97.40±5.84	5.40±1.57	0.80±0.20	1.20±0.37	(1)	(2)	1.00±0.51
Group VI	92.00±3.97	1.80±0.73	(1)	(1)	1.20±0.73	1.20±0.58	(1)

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001
Digits in parenthesis are number of yolky follicle in respective group
Since number was low, so mean could not be calculated.

Table 50: Mean category range (mm) of ovarian yolky follicles diameter in White Leghorn layer birds at 67th week of age slaughtered on 15th day of after withdrawal of restraint feeding and zinc administration (Batch-II)

	1-5mm	5.1-10mm	10.1 -15mm	15.1-20mm	20.1-25mm	25.1-30mm	30.1-35 mm
Group I	4.07±0.30	6.32±0.49	13.35±0.85	17.81±0.74	22.74±.59	28.11±0.95	32.38±0.71
Group II	4.04±0.28	6.07±0.24	12.20±0.62	16.90 (1)	23.18 (2)	26.14 (1)	31.34±1.08
Group III	4.42±0.21	6.67±0.66	13.45±0.96	15.90±0.10	22.89 (1)	28.24 (2)	33.71±0.49
Group VI	4.18±0.23	6.16±0.39	12.67 (1)	16.86 (1)	23.21±0.59	27.35±0.69	30.65 (1)

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001
Digits in parenthesis are number of yolky follicle in respective group

Table 51: Mean non yolky follicular diameter (µm) in White Leghorn layer birds at 67th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Mean Follicular Diameter Category Range (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group I	150.30±4.00	304.59±6.97	499.08±7.65	699.96±9.66	885.48±12.11	1303.90±57.81
Group II	141.30±3.00	286.45±7.16	482.90±8.83	678.53±11.22	876.28±16.78	1227.22±35.60
Group III	150.30±4.47	303.32±6.74	491.25±7.50	684.17±8.19	877.47±21.88	1292.40±58.89
Group IV	142.30±3.36	296.33±5.19	489.60±5.04	682.47±8.10	877.60±12.34	1255.17±47.55

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 52: Mean non yolky oocytes diameter (µm) category range in White Leghorn layer at 67th week of age, slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Oocytes Diameter Category Range (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group I	110.27±3.11	232.79±7.21	407.27±8.63	594.58±12.08	762.77±10.13	1136.16±55.03
Group II	101.47±2.24^{a***}	217.57±4.98	387.54±8.42	576.77±10.16^{a****}	757.48±13.49	1011.59±35.77
Group III	108.15±2.94^{b*}	231.40±5.71	395.53±6.22	575.46±7.88^{a****}	771.19±20.50	1118.78±60.16
Group IV	106.58±3.20	231.30±4.45	393.23±4.93	579.13± 8.72^{a****}	759.59±10.91	1112.42±44.56

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 53: Mean non yolky follicular wall thickness (µm) category range in White Leghorn layer birds at 67th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Follicular Wall Thickness Category Range (µm)					
	≤20µm	**21-40µm**	**41-60µm**	**61-80µm**	**81-100µm**	**>101µm**
Group I	17.68±0.39	32.41±0.27	47.52±0.74	66.58±0.80	90.11±1.03	130.92±6.03
Group II	13.62±0.27^{a****}	27.88±0.48^{a****}	46.69±0.73	64.45±1.11	84.99±0.82^{a***}	103.57±2.31^{a****}
Group III	13.56±0.31^{a****}	29.06±0.55^{a****}	46.66±0.68	65.85±0.85	88.43±1.34^{b*}	129.01±7.07^{b***}
Group IV	13.61±0.22^{a****}	28.41±0.49^{a****}	46.63±0.45	65.22±0.74	86.84±1.03^{a*}	128.06±3.98^{b****}

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

Table 54: Mean number of non yolky follicles per section of ovary in different category range in White Leghorn layer birds at 67th week of age slaughtered on 15th day after withdrawal of restraint feeding and zinc administration (Batch-II)

	Mean Follicular Number Category Range (µm)					
	≤200µm	**201-400µm**	**401-600µm**	**601-800µm**	**801-1000µm**	**>1001µm**
Group-I	27.20±3.02	9.20±1.28	6.60±1.08	3.60±1.03	1.40±0.51	1.60±0.51
Group-II	22.40±3.70	8.20±2.08	7.40±1.60	4.00±1.34	1.80±0.73	1.40±0.51
Group-III	25.60±3.85	9.80±1.74	8.00±1.30	4.20±1.07	2.80±1.02	2.00±0.55
Group-IV	24.80±4.32	9.00±1.58	7.60±0.93	4.40±1.08	2.20±0.86	1.80±0.58

Values are means±SEM (N=5)
a=Group-I compared to all other groups.
b=Group-II compared to Group-III and group-IV.
c=Group-III compared to Group-IV
*=0.05 **=0.02 ***=0.01 and ****=0.001

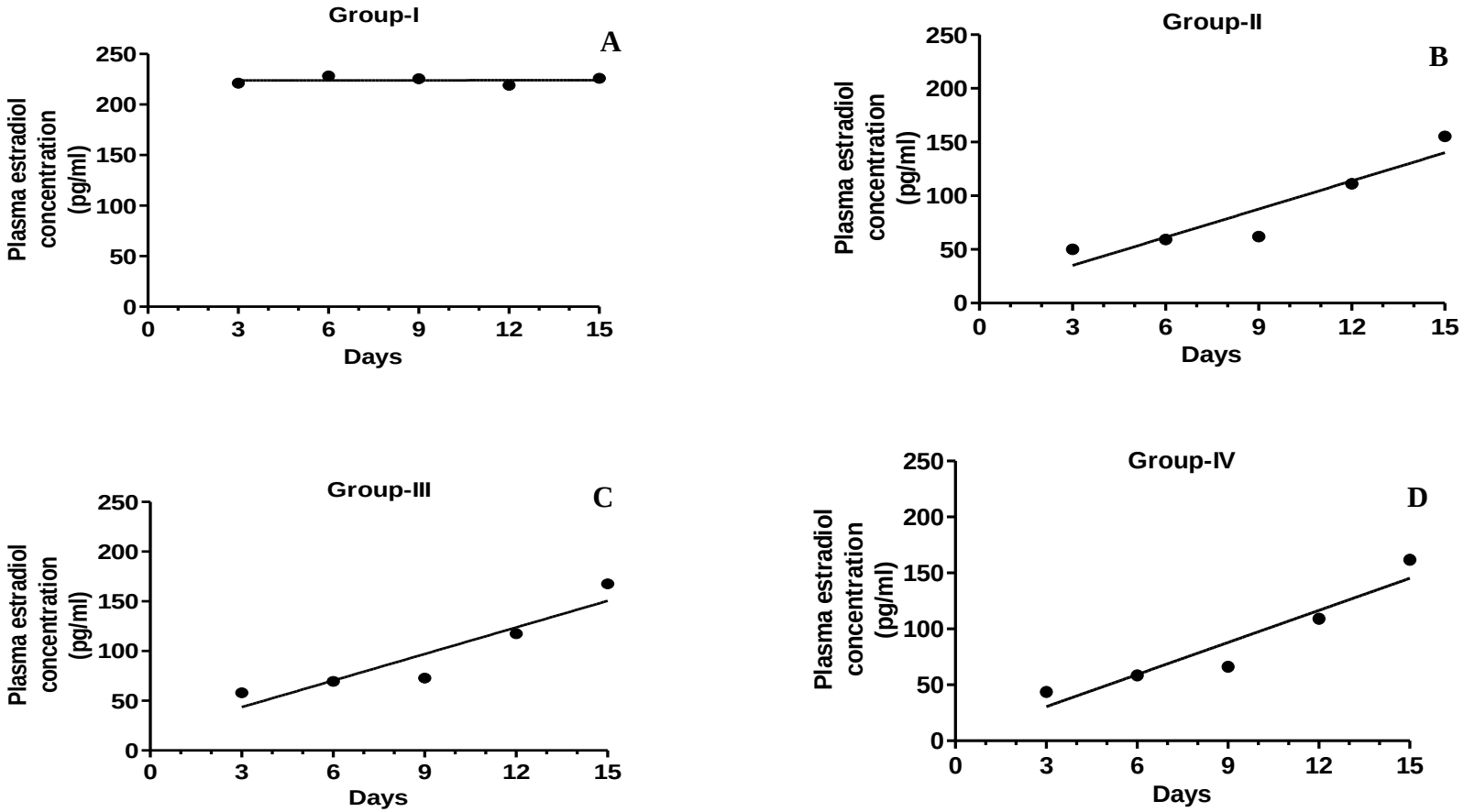


Fig. 49: Regression line showing non significant increase in mean plasma estradiol levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during fifteen days after withdrawal of treatment in White Leghorn layer birds at 67th week of age (Batch-II).

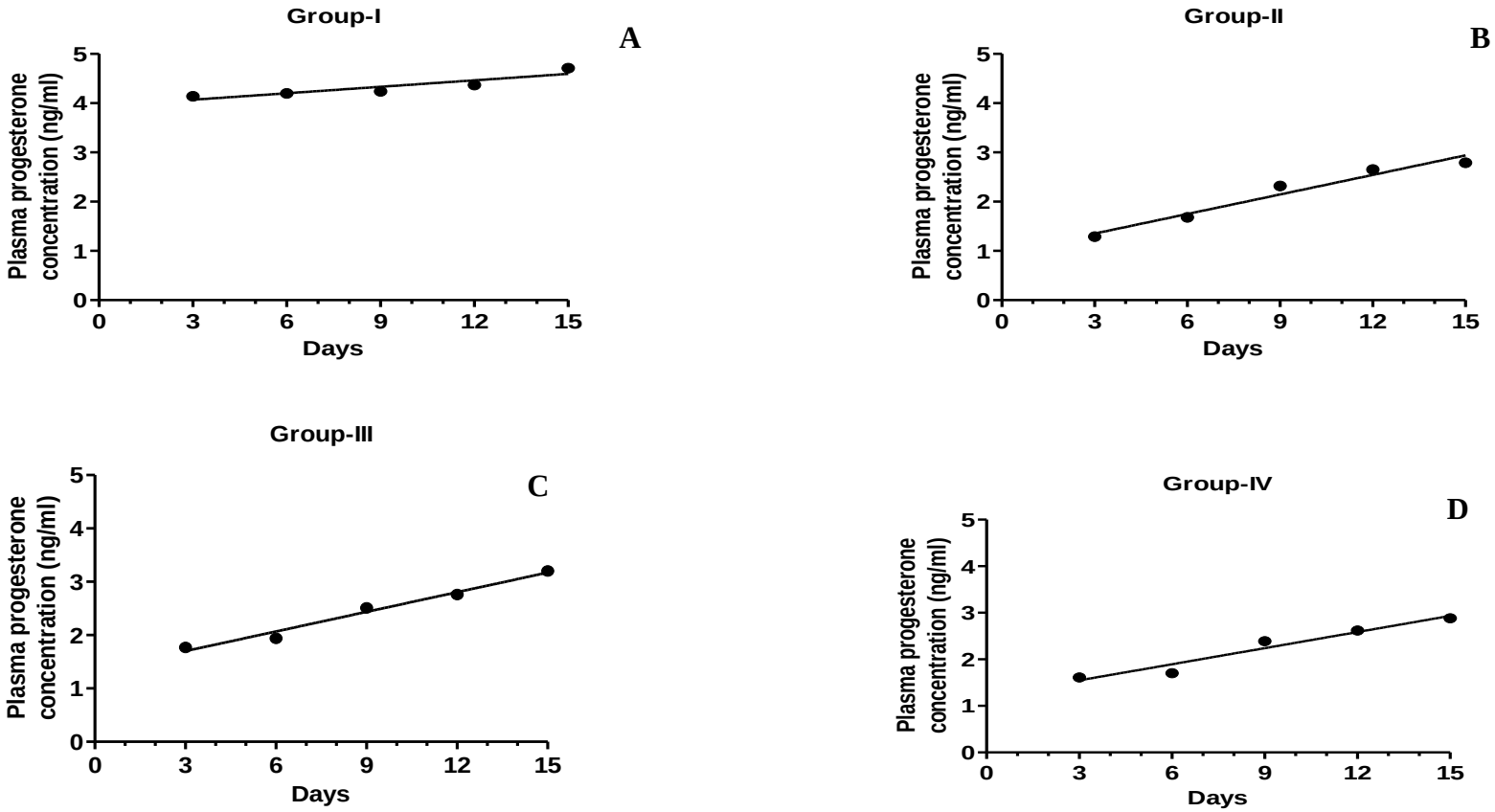


Fig. 50: Regression line showing non significant increase in mean plasma progesterone levels in treated groups compared to control birds (A) Birds fed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during fifteen days after withdrawal of treatment in White Leghorn layer birds at 67th week of age (Batch-II).

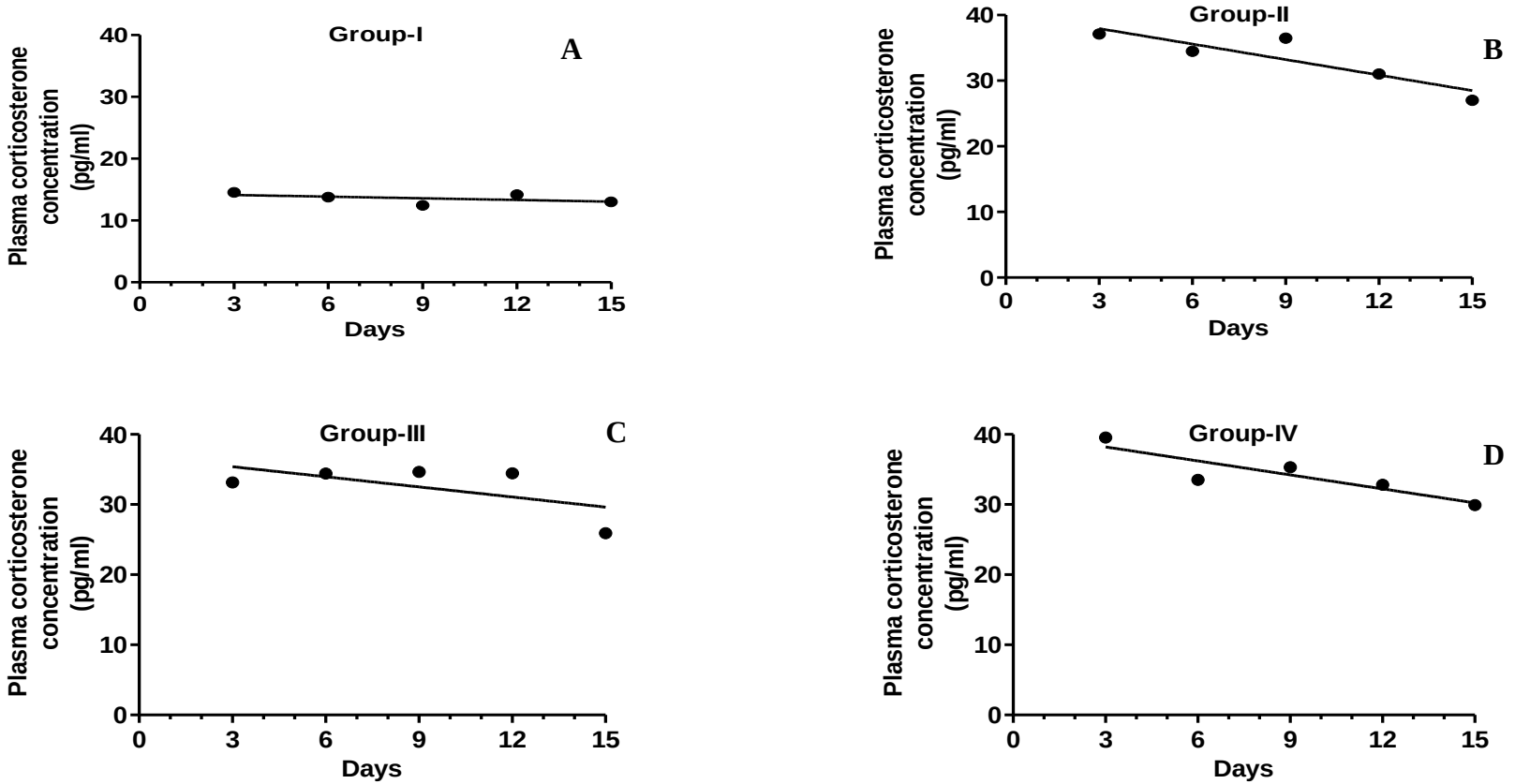


Fig. 51: Regression line showing non significant decrease in mean plasma corticosterone levels in treated groups compared to control birds (A) Birds feed normal diet (B) Birds Restraint feeding (C) Fed 25,000ppm zinc/Kg feed (D) Fed 30,000ppm zinc/Kg feed during fifteen days after withdrawal of treatment in White Leghorn layer birds at 67th week of age (Batch-II).

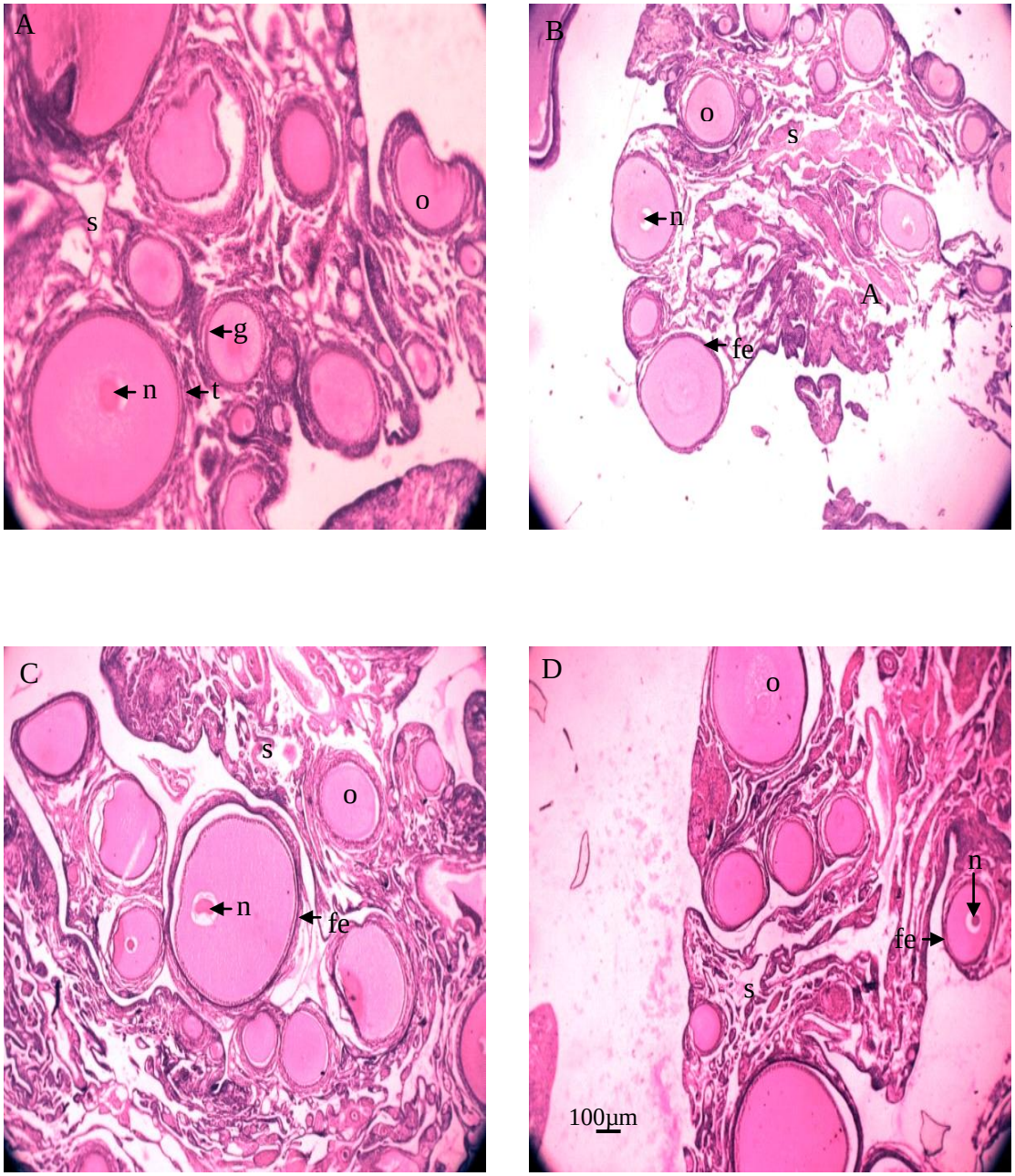


Fig. 38: Photomicrograph of the middle portion of the cross section of ovary showing follicles in control and treatment groups, birds slaughtered on 12th day treatment at 67th week of age (Batch-I). (A) Control showing greater number of large and small follicles (o) oocyte, (t) thecal layer, (g) granulosa layer, (s) stromal tissue, (n) nucleus (B) Group-II (off fed) and zinc treated groups (C) and (D) (Group-III and Group-IV) showing decrease in number of follicles (o) oocyte, (fe) follicular epithelium, (g) granulosa layer, (s) stromal tissue, (n) nucleus, greater decrease in Group-II and Group-IV. H & E.

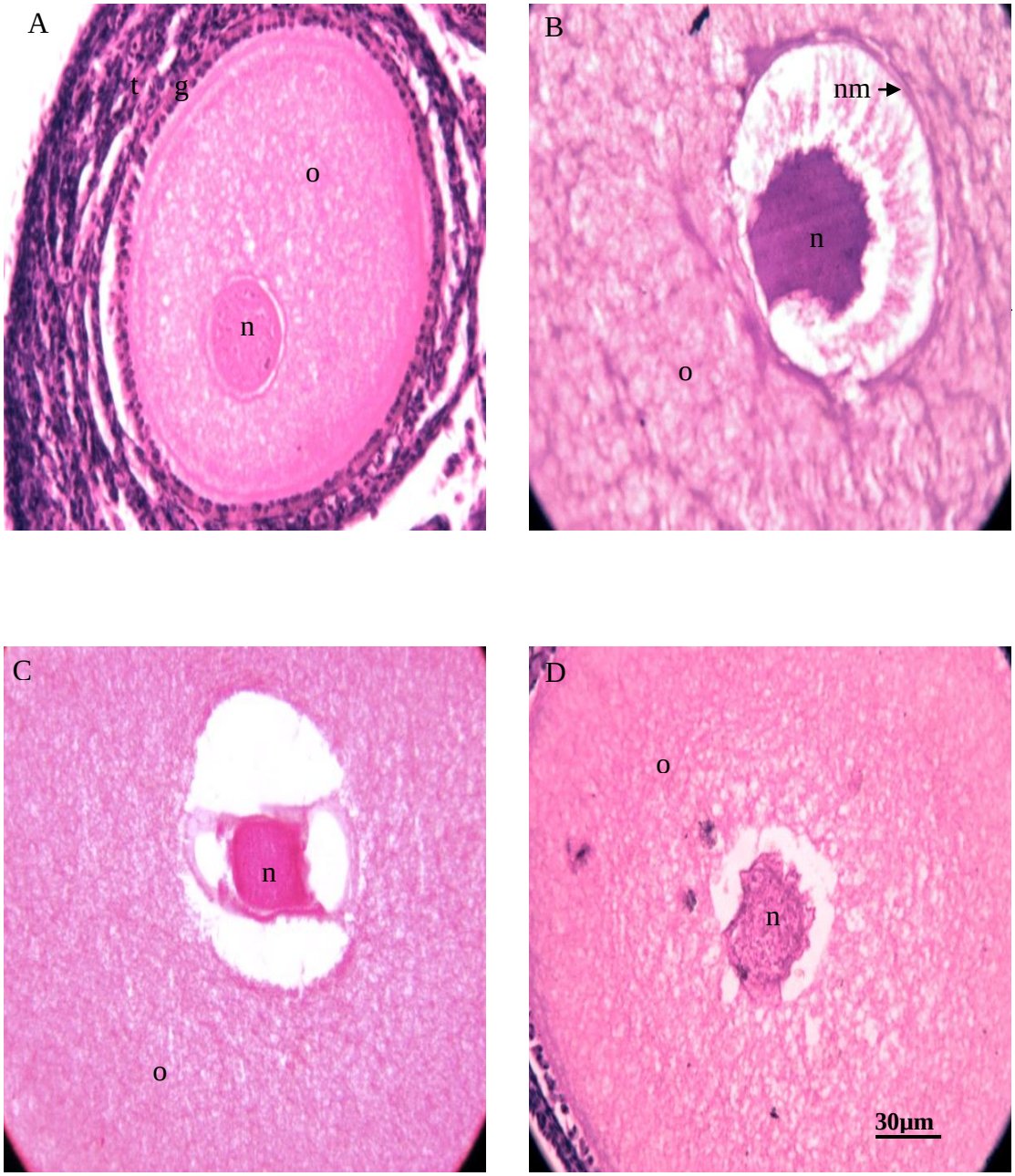


Fig. 39: Photomicrograph of the middle portion of the cross section of ovary showing oocyte nucleus in control and treatment groups, birds slaughtered on 12th day treatment at 67th week of age (Batch-I). (A) Control showing normal spherical nucleus with clear nuclear membrane (o) oocyte, (n) nucleus (B) Group-II (off fed) (C) Group-III and (D) Group-IV (zinc treated groups) showing abnormal and deshaped nucleus with unclear nuclear membrane (o) oocyte, (nm) nuclear membrane and (n) nucleus. H & E.

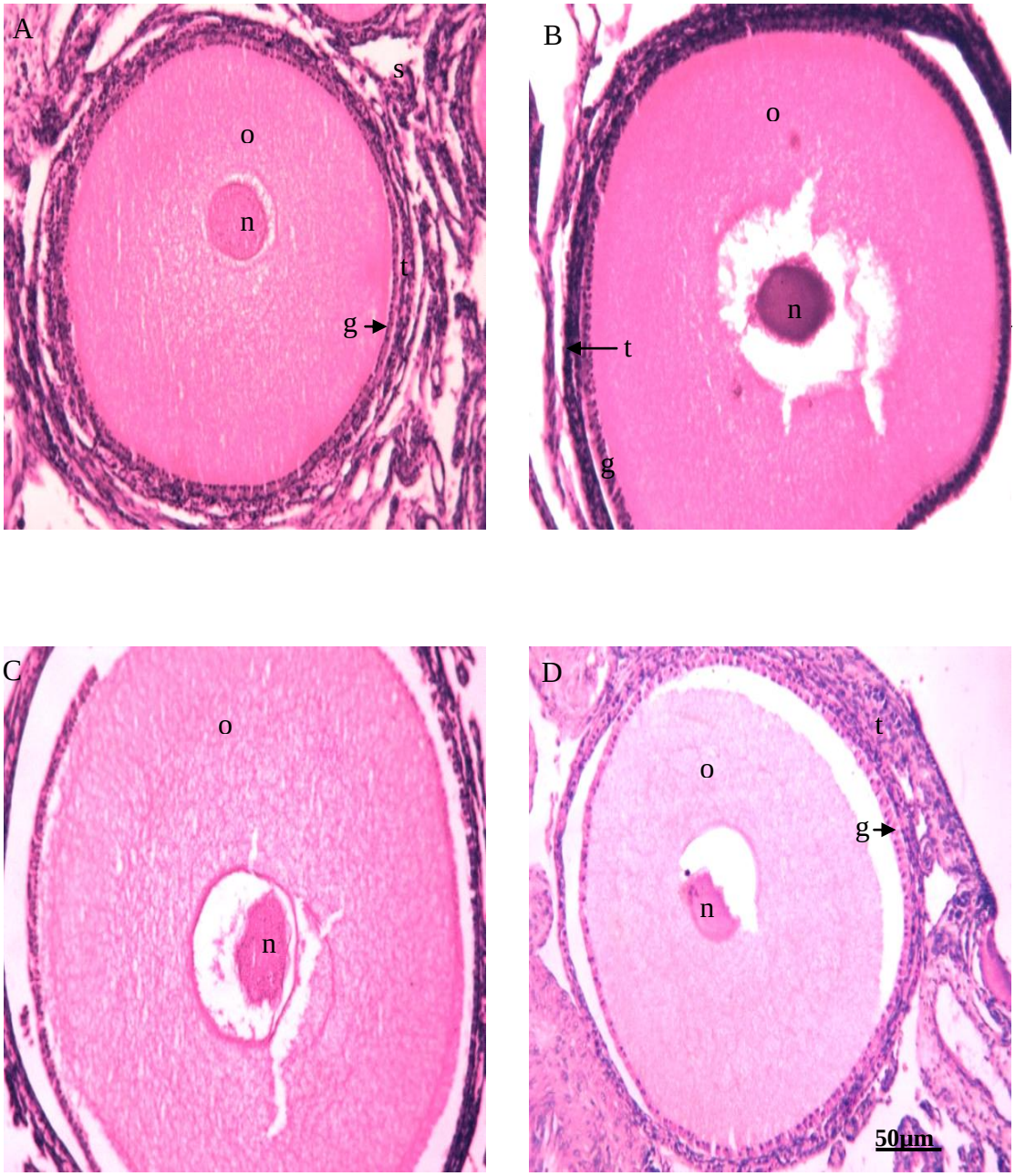


Fig. 40: Photomicrograph of the middle portion of the cross section of ovary showing cytoplasm (ooplasm) of oocyte in control and treatment groups, birds slaughtered on 12th day treatment at 67th week of age (Batch-I). (A) Control showing normal cytoplasm and intact thecal and granulosa layers (s) stroma (o) oocyte, (t) thecal layer, (g) granulosa layer, (B) Group-II (off fed) (C) Group-III and (D) Group-IV (zinc treated groups) showing abnormal cytoplasm, more disintegration in Group-II and Group-IV (o) oocyte, (t) thecal layer and (g) granulosa layer. H & E.

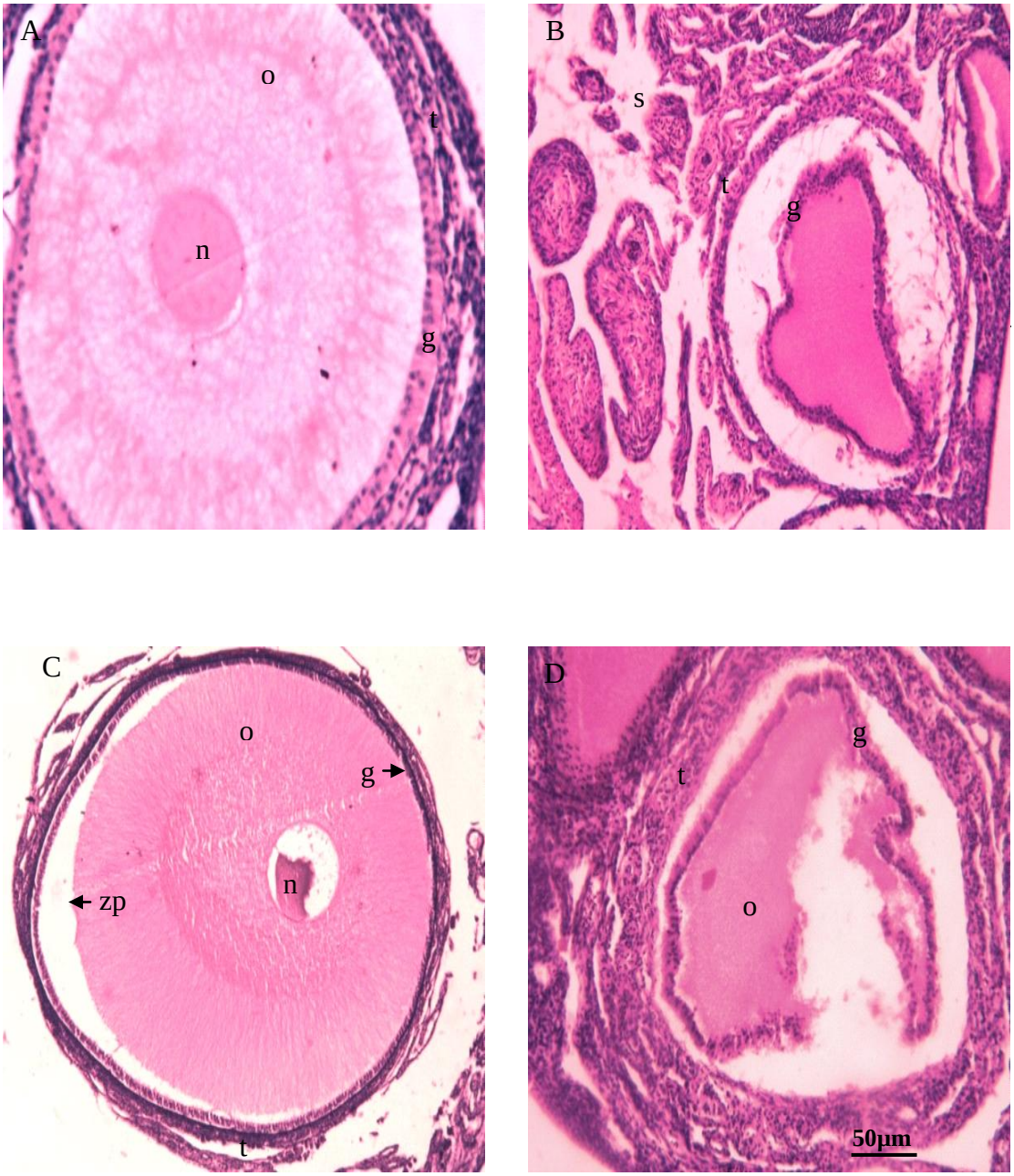


Fig. 41: Photomicrograph of the middle portion of the cross section of ovary showing changes in zona pellucida in control and treatment groups, birds slaughtered on 12th day treatment at 67th week of age (Batch-I). (A) Control showing normal zona pellucida attached with granulosa layer (o) oocyte, (t) thecal layer, (g) granulosa layer, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing disrupted zona pellucida layer (zp) zona pellucida, (o) oocyte, (t) thecal layer, (g) granulosa layer, (zp) zona pellucida and (n) nucleus. H & E.

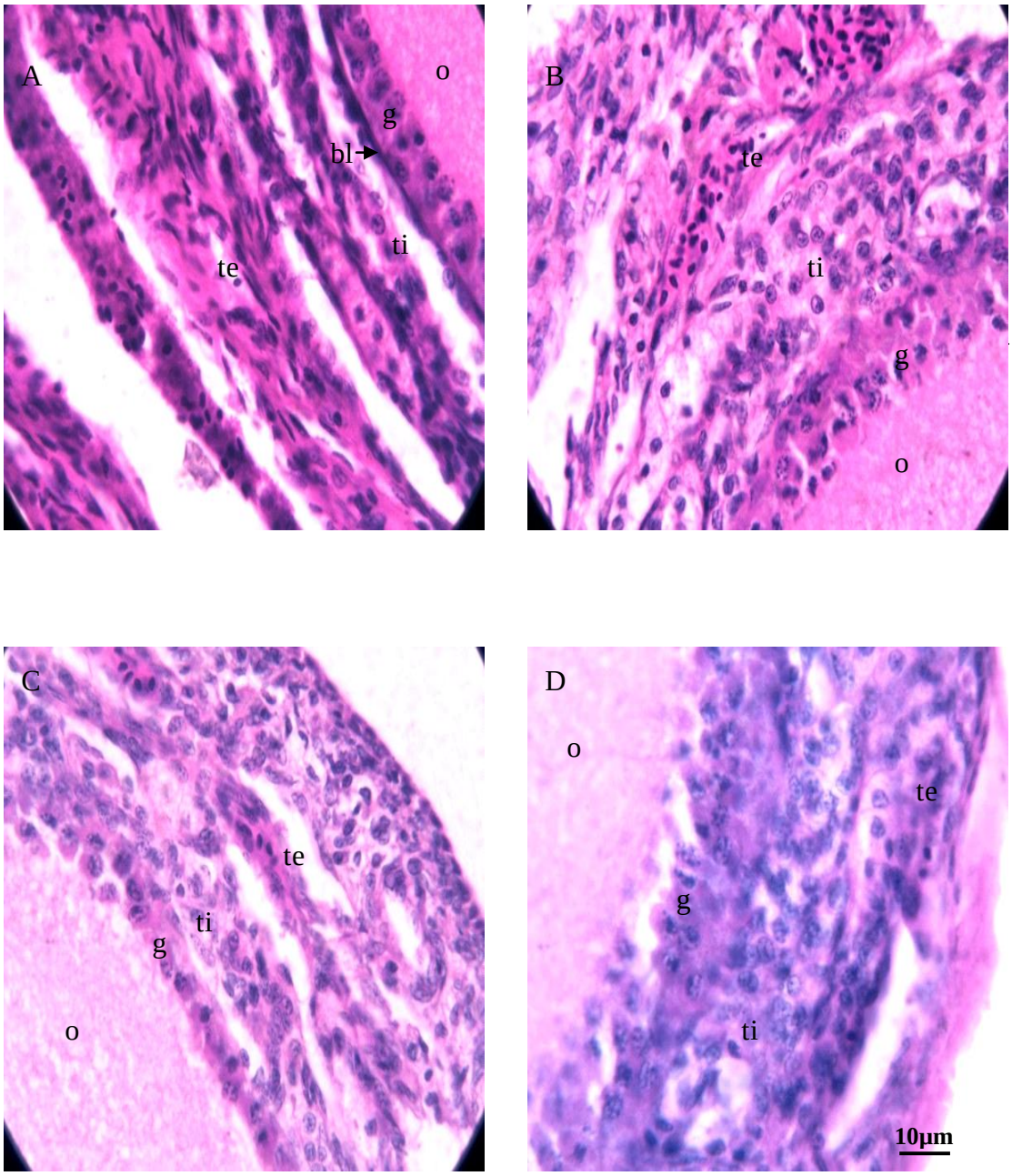


Fig. 42: Photomicrograph of the middle portion of the cross section of ovary showing granulosa and basal lamina in control and treatment groups, birds slaughtered on 12th day treatment at 67th week of age (Batch-I). (A) Control clear granulosa with basal lamina and thecal layer, (ti) theca interna, (g) granulosa, (bl) basal lamina, (te) theca externa (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing rough and intermingled basal and granulosa, (ti) theca interna, (g) granulosa, (bl) basal lamina and (te) theca externa. H & E.

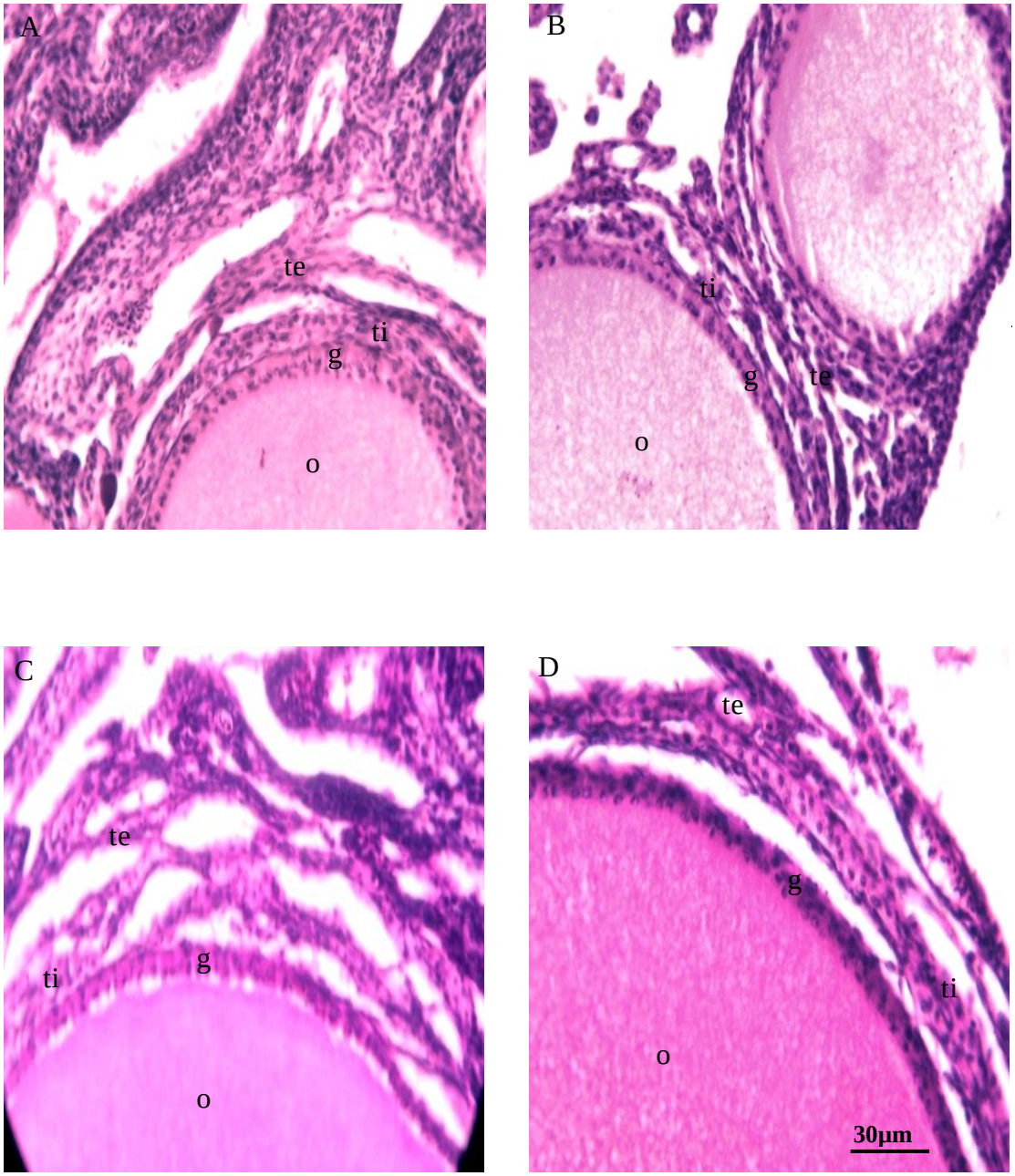


Fig. 43: Photomicrograph of the middle portion of the cross section of ovary showing thickness of follicular wall in control and treatment groups birds slaughtered on 12th day treatment at 25th week of age (Batch-I). (A) Control showing greater thickness of follicular layer, (ti) theca interna, (g) granulosa, (o) oocyte, (te) theca externa, (B) Group-II (off fed), (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing reduction in thickness of follicular wall, more in Group-II and Group-IV (ti) theca interna, (g) granulosa, (o) oocyte and (te) theca externa. H & E.

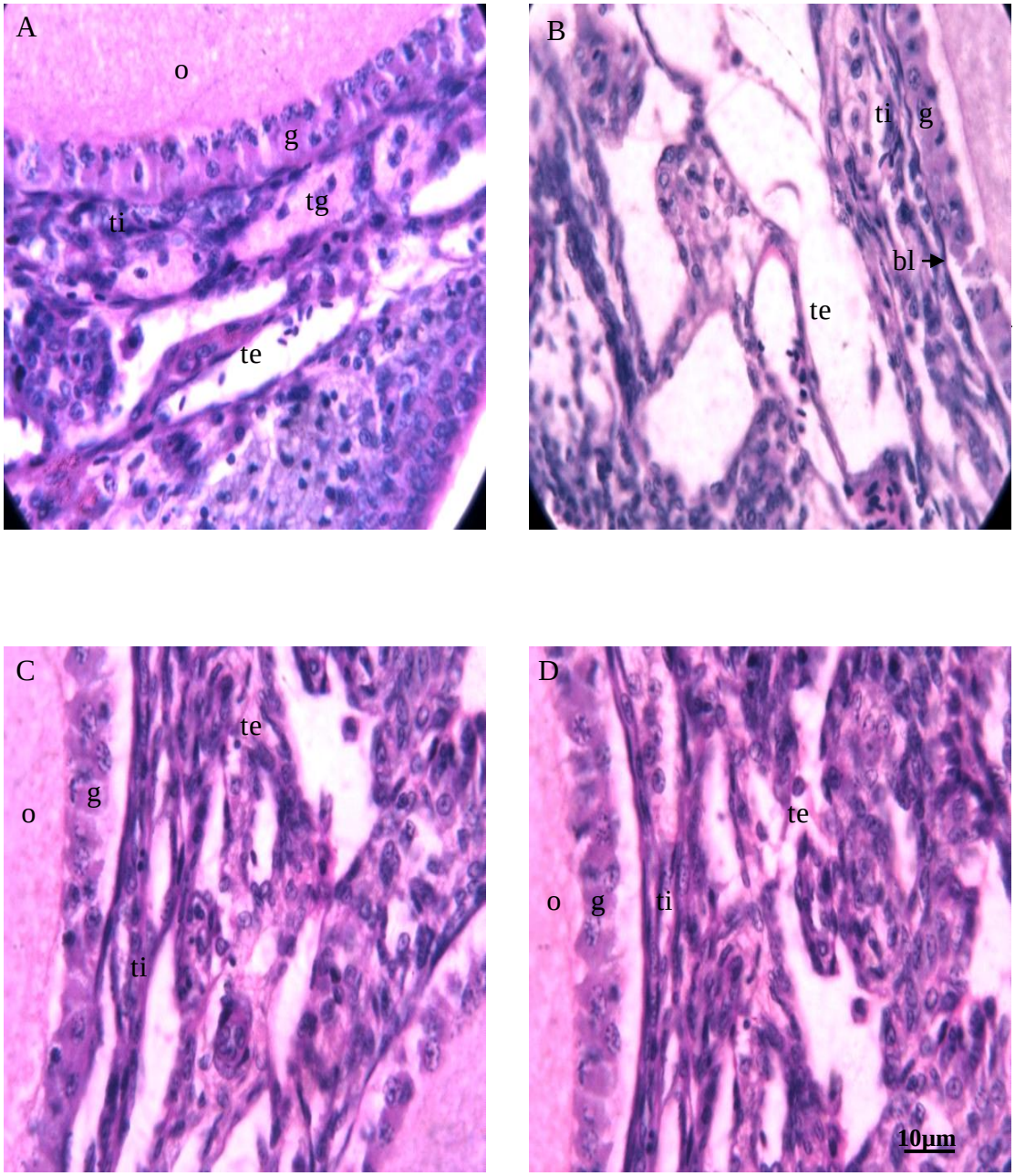


Fig. 44: Photomicrograph of the middle portion of the cross section of ovary showing thecal gland concentration in control and treatment groups birds slaughtered on 12th day treatment at 67th week of age (Batch-I). (A) Control showing greater number of thecal glands in thecal layer, (tg) thecal gland, (g) granulosa, (o) oocyte, (ti) theca interna (te) theca externa, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing less number of thecal gland in thecal layer (tg) thecal gland, (g) granulosa, (o) oocyte, (ti) theca interna (te) theca externa and (bl) basal lamina. H & E.

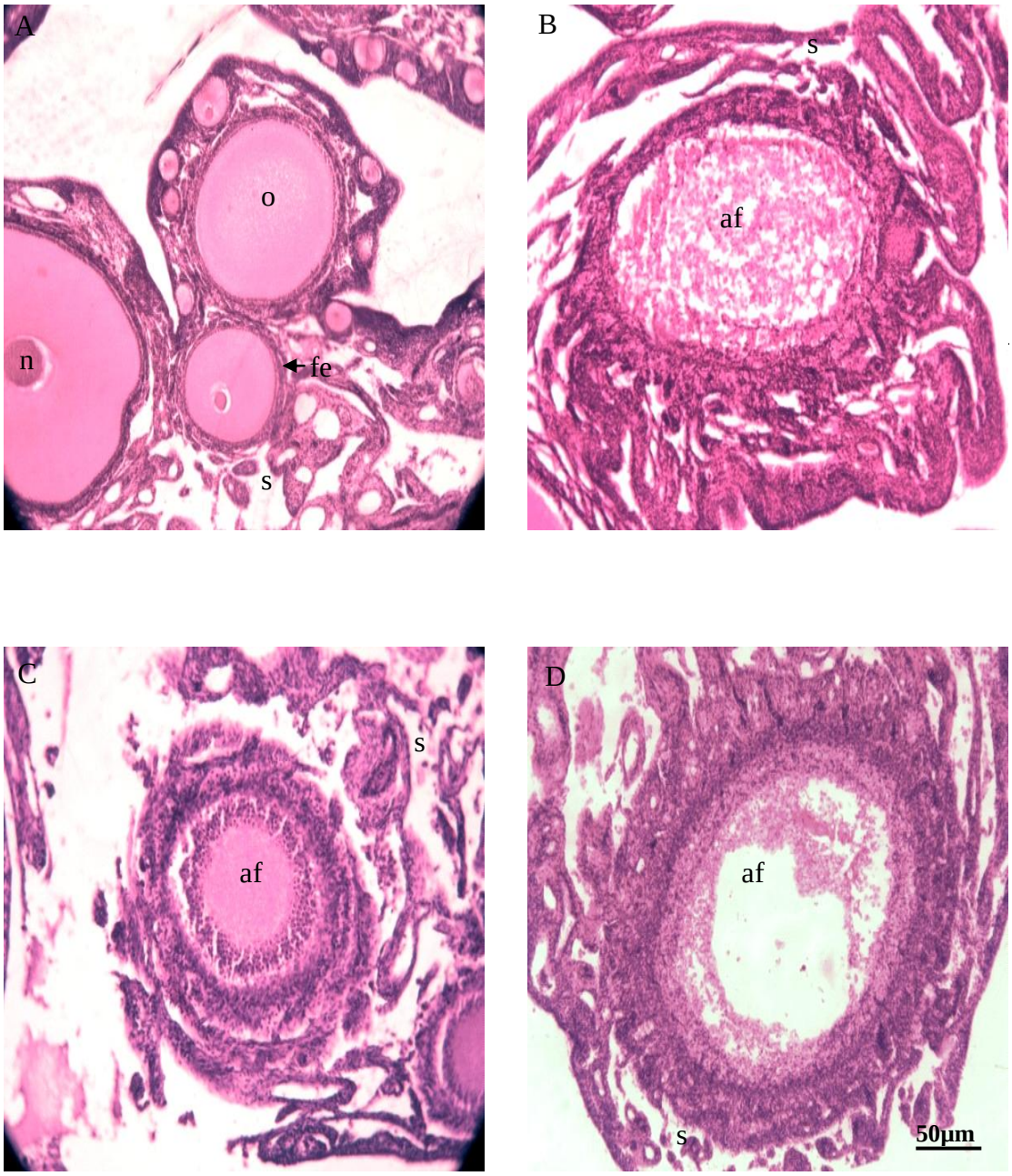


Fig. 45: Photomicrograph of the middle portion of the cross section of ovary showing atretic follicle in control and treatment groups birds slaughtered on 12th day treatment at 67th week of age (Batch-I). (A) Control showing no atretic follicle in follicle hierarchy, (fe) follicular epithelium (s) stroma, (o) oocyte, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing presence of atretic follicles (af) atretic follicle and (s) stroma. H & E.

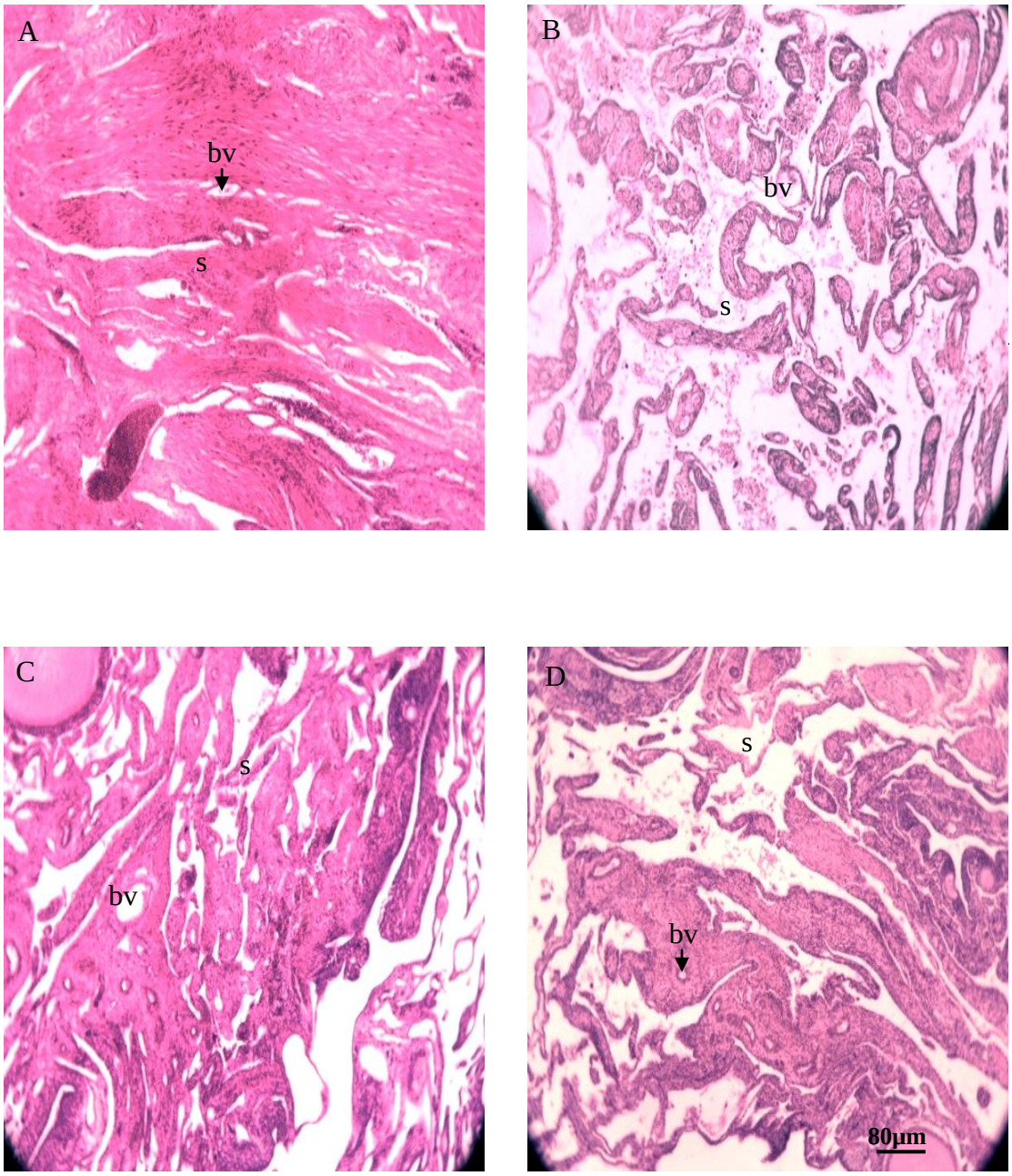


Fig. 46: Photomicrograph of the middle portion of the cross section of ovary showing stromal tissue organization in control and treatment groups birds slaughtered on 12th day treatment at 67th week of age (Batch-I). (A) Control showing compact organization of stromal tissue with numerous blood vessels (bv) blood vessels, (s) stroma, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) showing loose arrangement of stromal tissue (bv) blood vessel and (s) stroma. H & E.

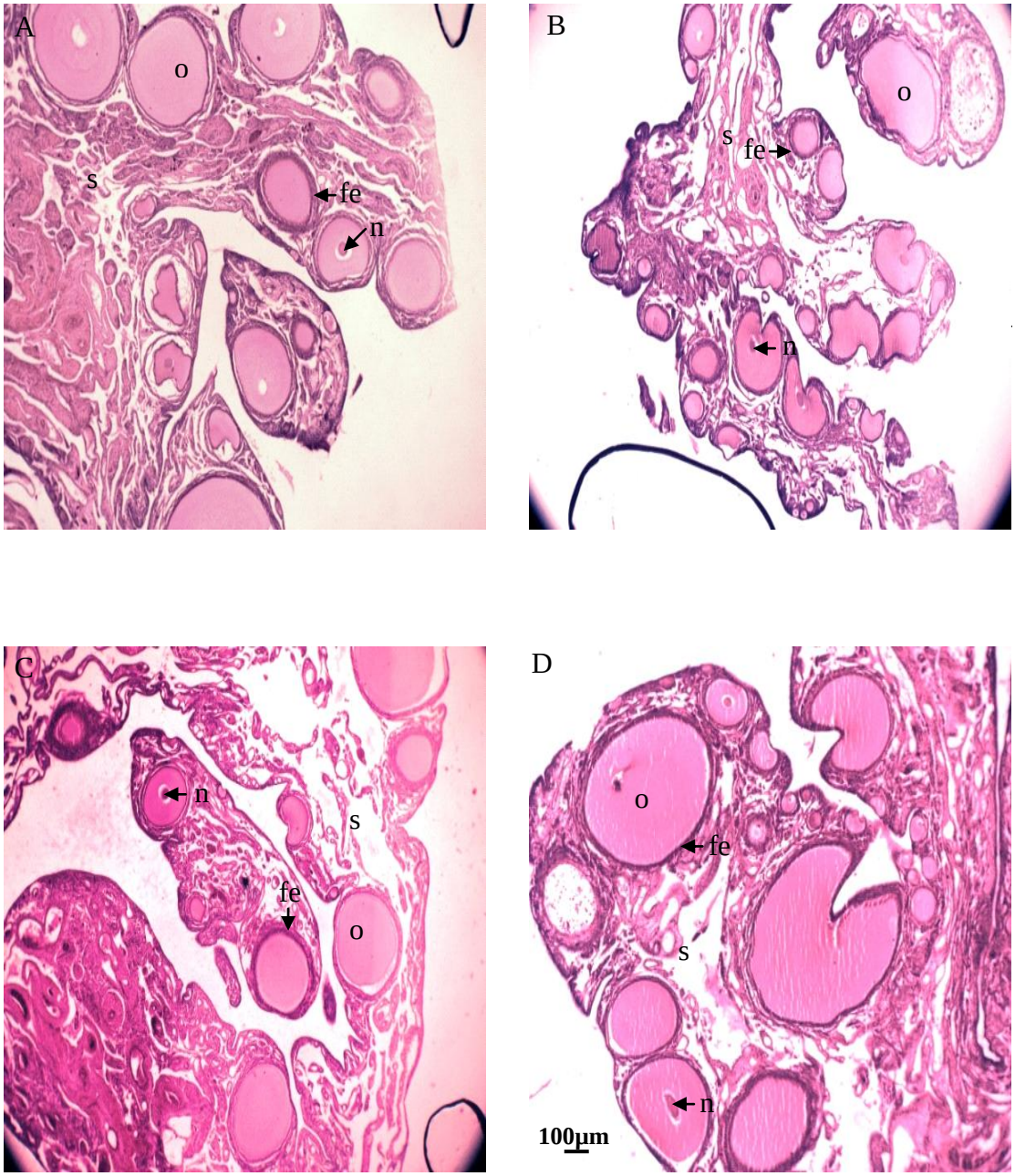


Fig 52: Photomicrograph of the middle portion of the cross section of ovary showing follicle in control and treatment groups, slaughtered on 15th day after withdrawal of treatment and resumption to normal feed at 67th week of age (Batch-II). (A) Control showing large and small follicles of all diameter categories (o) oocyte, (fe) follicular epithelium, (g) granulosa layer, (s) stromal tissue, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing large and small follicles of all diameter categories (o) oocyte, (fe) follicular epithelium, (g) granulosa layer, (s) stromal tissue and (n) nucleus. H & E.

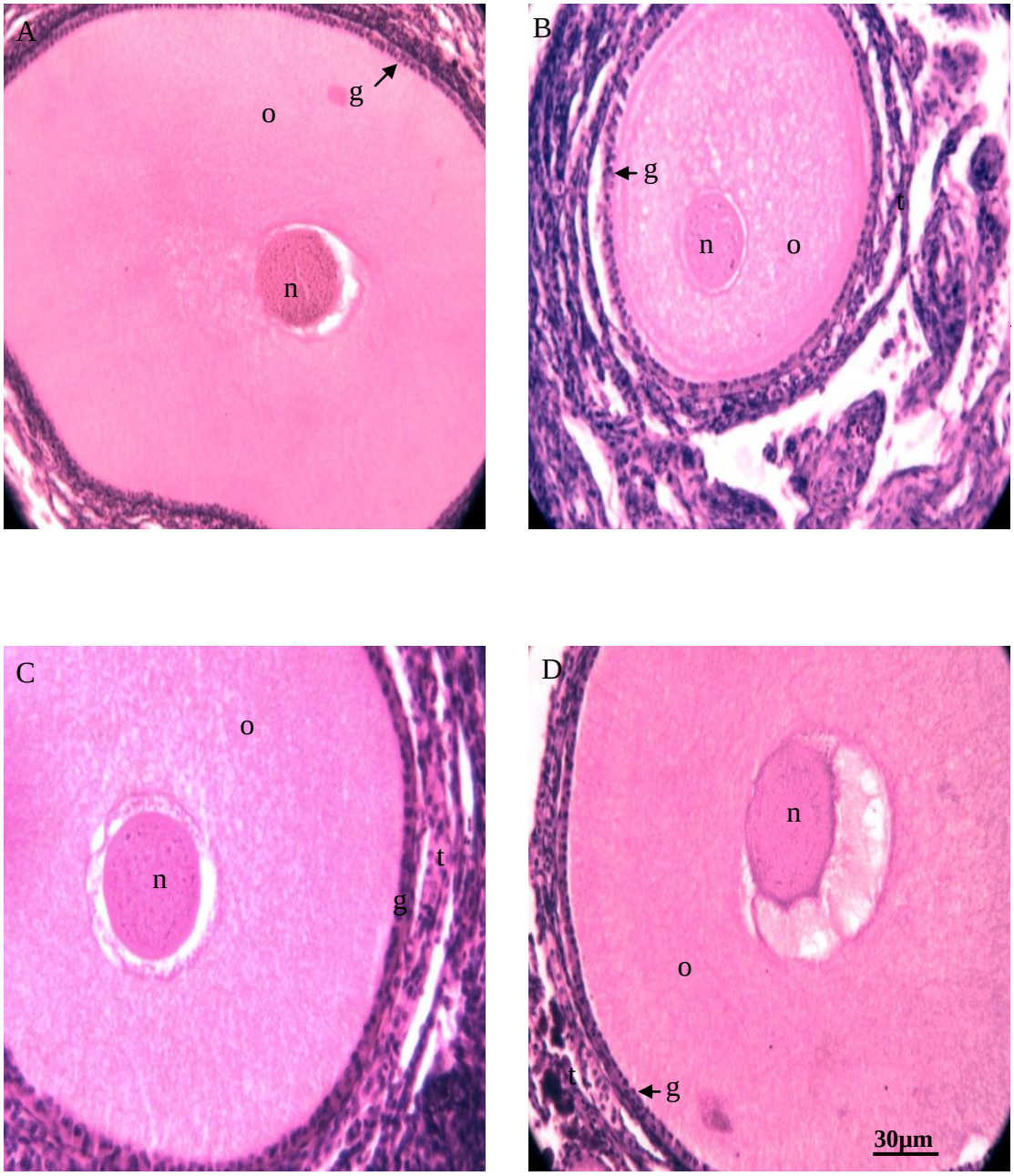


Fig 53: Photomicrograph of the middle portion of the cross section of ovary showing nucleus of follicle in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal feed at 67th week of age (Batch-II). (A) Control showing normal spherical nucleus with clear nuclear membrane (o) oocyte, (n) nucleus, (g) granulosa, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing normal spherical nucleus with clear nuclear membrane (o) oocyte, (g) granulosa, (t) thecal layer and (n) nucleus. H & E.

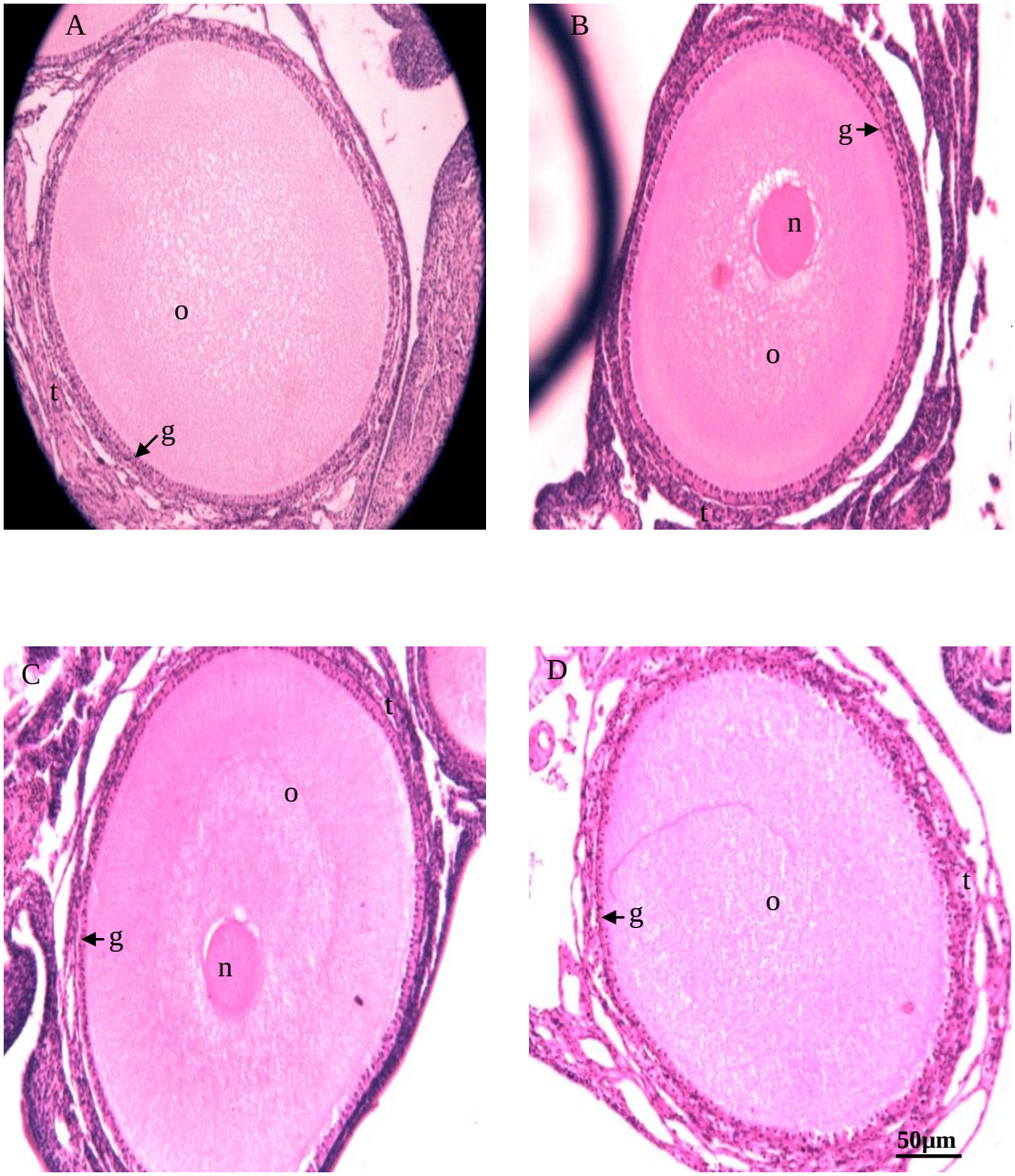


Fig 54: Photomicrograph of the middle portion of the cross section of ovary showing cytoplasm of follicle in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal feed at 67th week of age (Batch-II). (A) Control showing normal cytoplasm and intact thecal and granulosa layers (o) oocyte, (t) thecal layer, (g) granulosa layer, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing normal cytoplasm and intact thecal and granulosa layers (o) oocyte, (t) thecal layer, (g) granulosa layer and (n) nucleus. H & E.

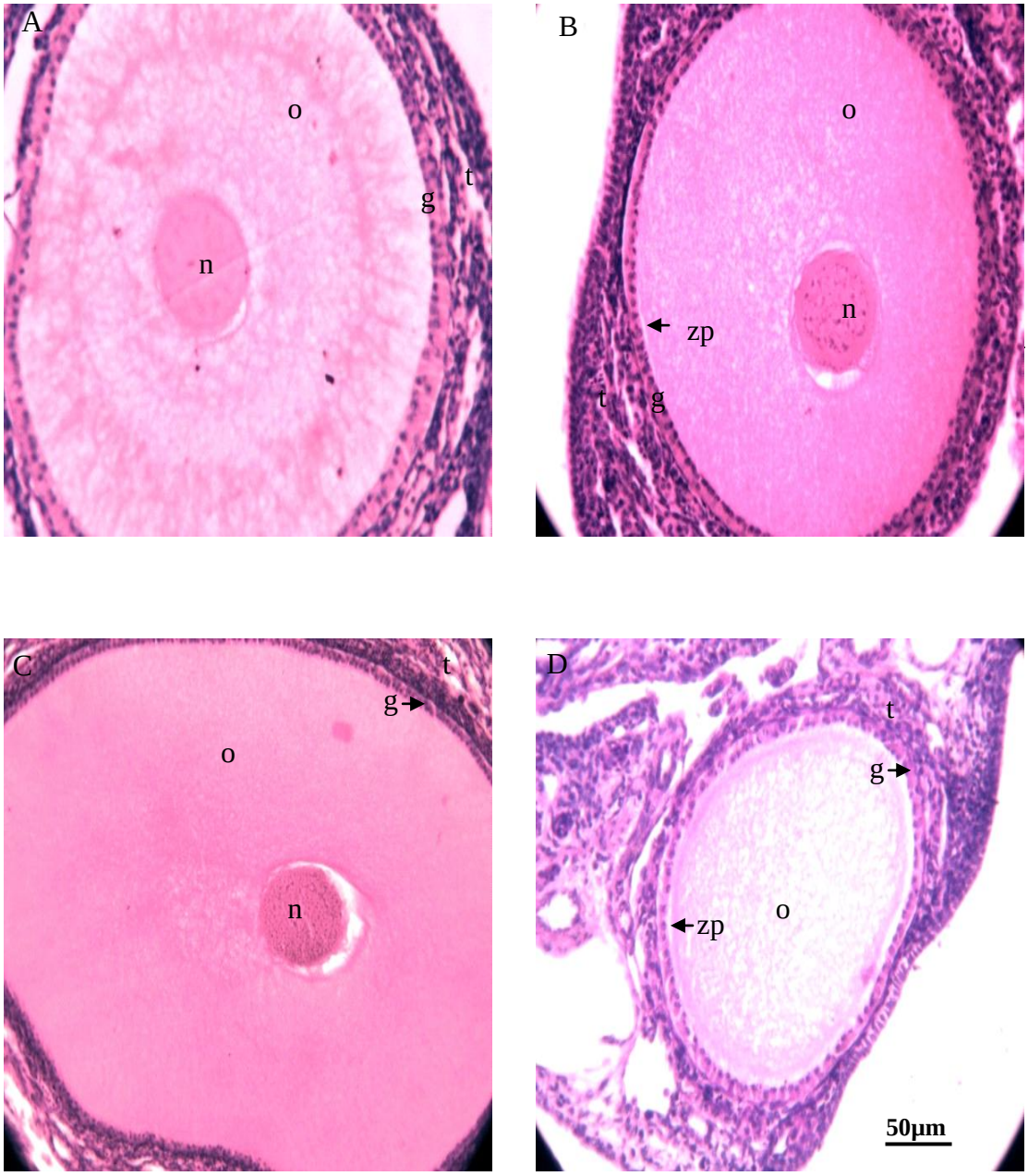


Fig 55: Photomicrograph of the middle portion of the cross section of ovary showing zona pellucida in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal feed at 67th week of age (Batch-II). (A) Control showing normal zona pellucida attached with granulosa layer (o) oocyte, (t) thecal layer, (g) granulosa layer, (n) nucleus, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing normal zona pellucida attached with granulosa layer (o) oocyte, (t) thecal layer, (g) granulosa layer, (n) nucleus and (zp) zona pellucida. H & E.

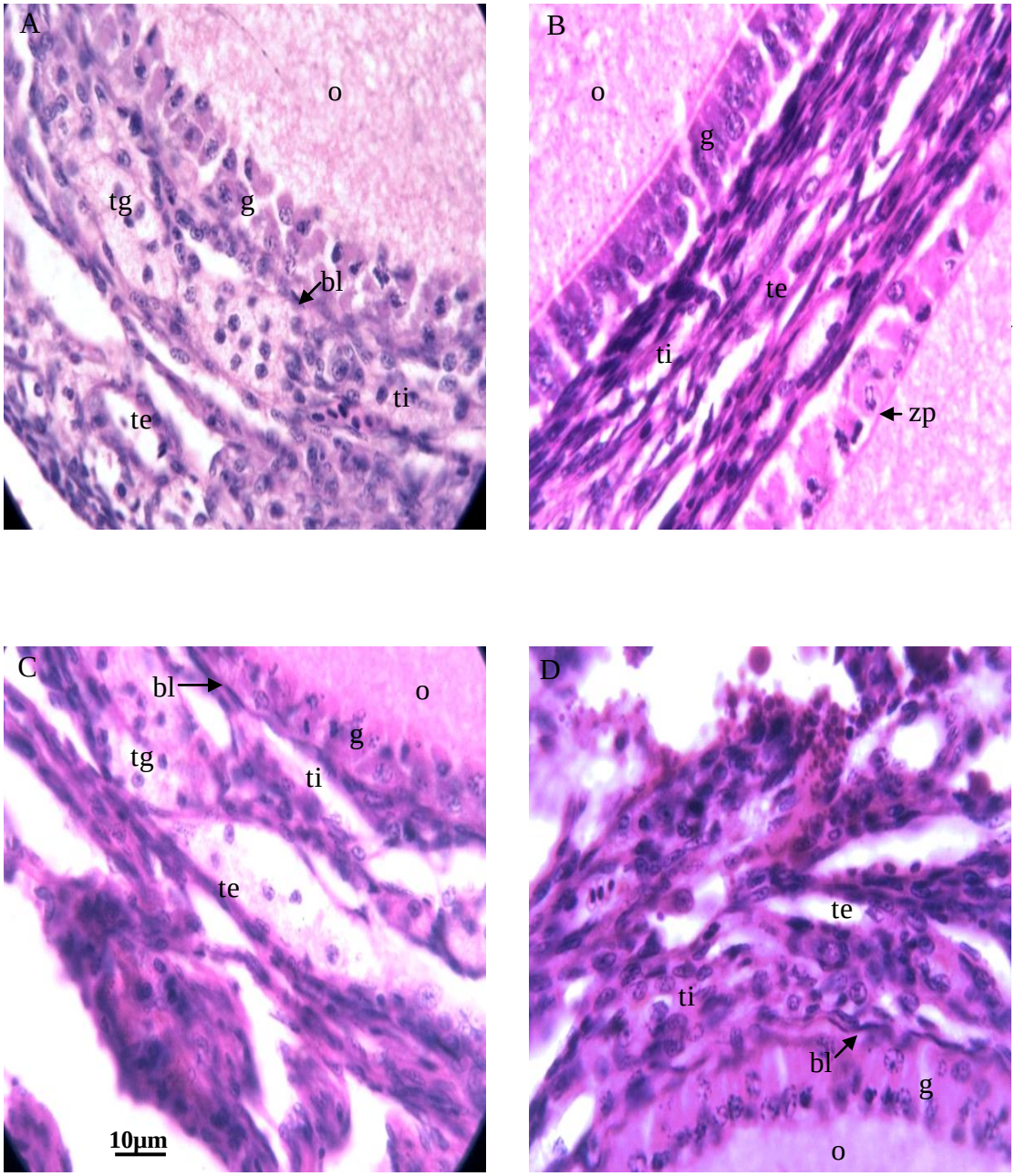


Fig 56: Photomicrograph of the middle portion of the cross section of ovary showing granulosa and basal lamina in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 67th week of age (Batch-II). (A) Control clear granulosa with basal lamina and thecal layer, (ti) theca interna, (te) theca externa (g) granulosa, (bl) basal lamina, (tg) thecal gland, (o) oocyte, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing clear granulosa with basal lamina and thecal layer , (ti) theca interna, (te) theca externa, (g) granulosa, (bl) basal lamina, (tg) thecal gland, (o) oocyte and zona pellucida. H & E.

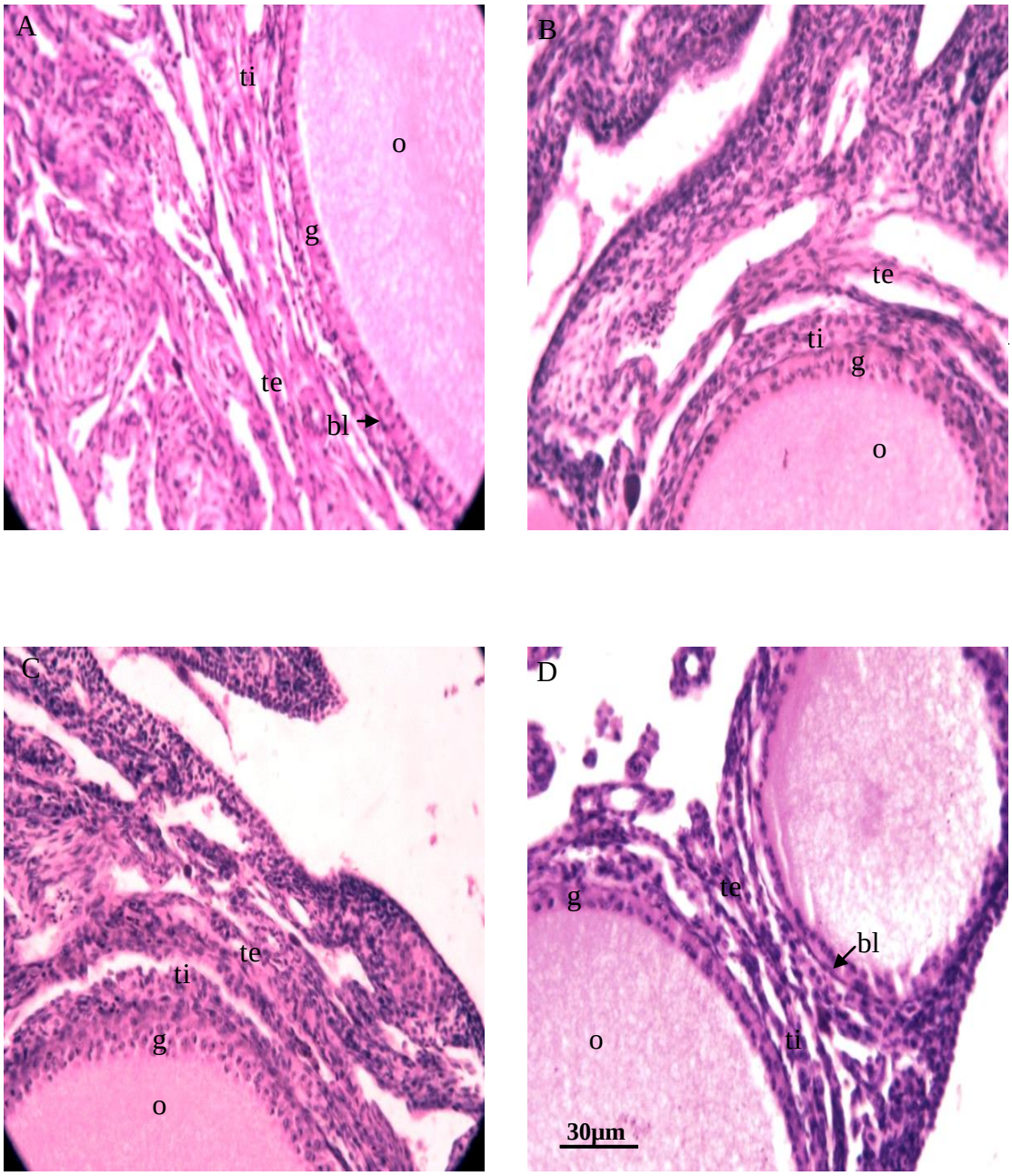


Fig 57: Photomicrograph of the middle portion of the cross section of ovary showing thickness of thecal layer in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 67th week of age (Batch-II). (A) Control showing greater thickness of follicular layer, (bl) basal lamina, (g) granulosa, (o) oocyte, (ti) theca interna, (te) theca externa, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing increased thickness of follicular layer (bl) basal lamina, (g) granulosa, (o) oocyte, (ti) theca interna and (te) theca externa. H & E.

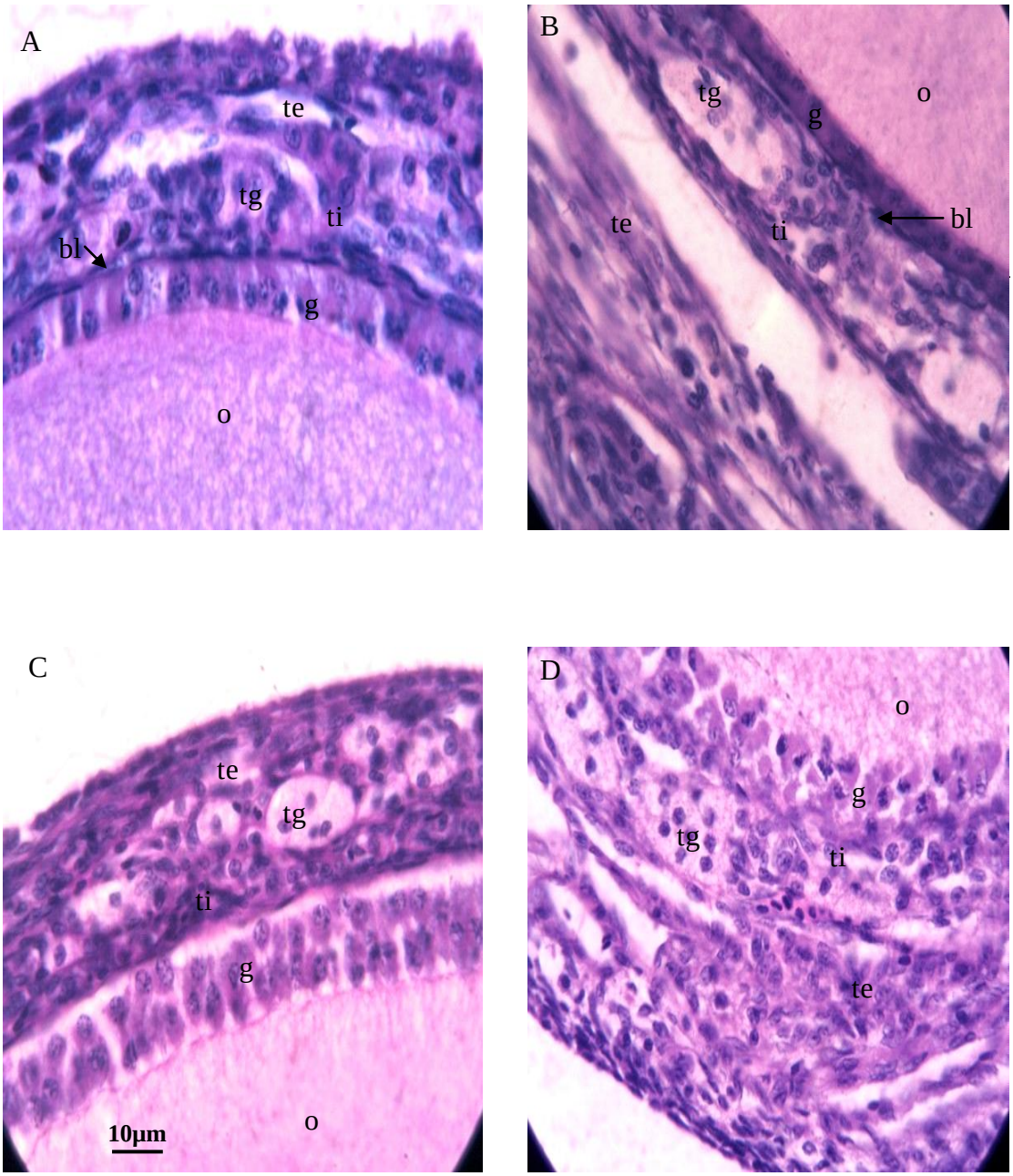


Fig 58: Photomicrograph of the middle portion of the cross section of ovary showing thecal gland concentration in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 67th week of age (Batch-II). (A) Control showing greater number of thecal gland in thecal layer, (tg) thecal gland, (g) granulosa, (o) oocyte, (ti) theca interna, (te) theca externa, (bl) basal lamina, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing increased number of thecal gland in thecal layer (tg) thecal gland, (g) granulosa, (o) oocyte, (t) thecal layer and (bl) basal lamina. H & E.

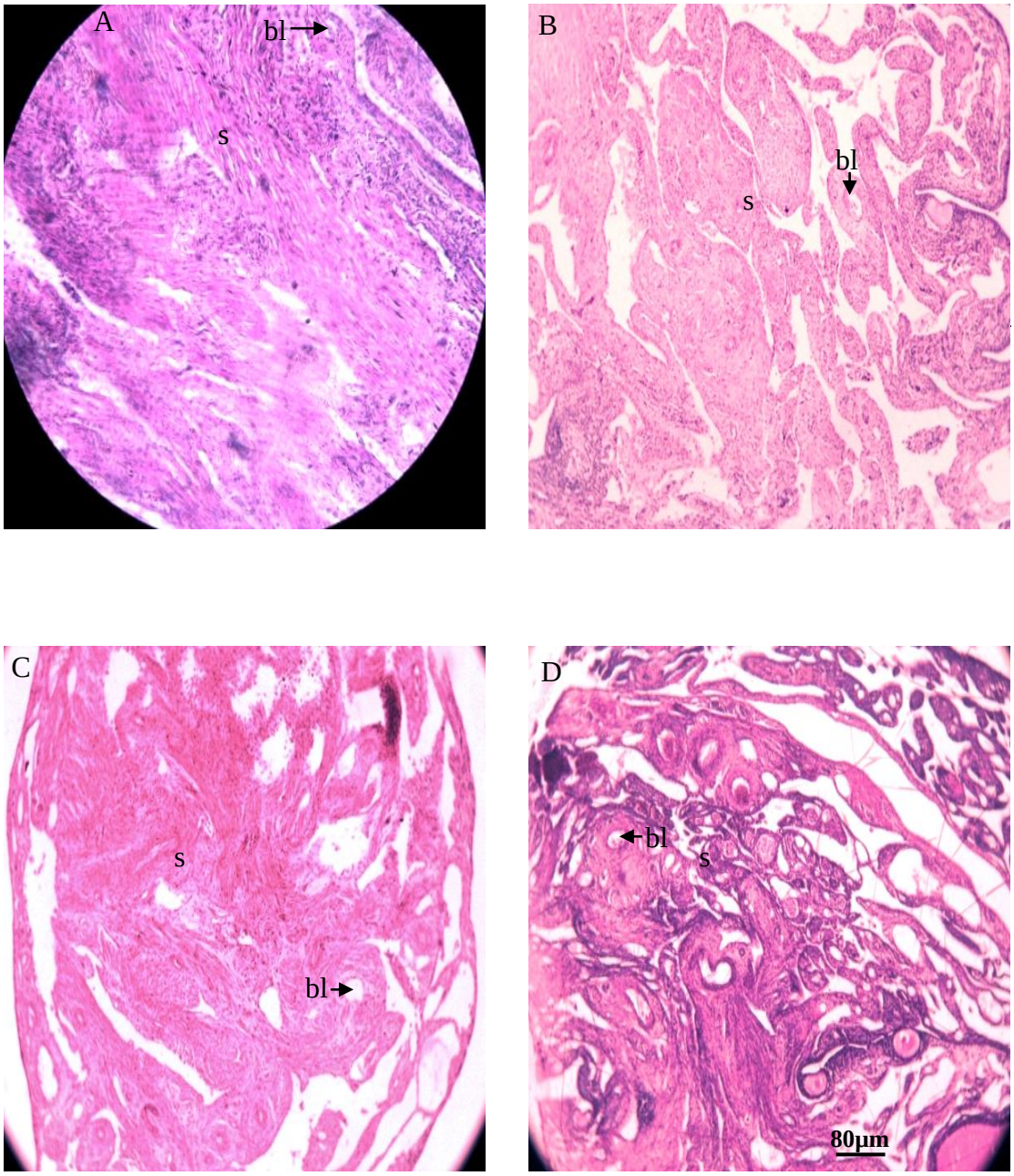


Fig 59: Photomicrograph of the middle portion of the cross section of ovary showing stromal tissue organization in control and treatment groups birds slaughtered on 15th day after withdrawal of treatment and resumption to normal at 67th week of age (Batch-II). (A) Control showing compact organization of stroma tissue with numerous blood vessels (bv) blood vessels, (s) stroma, (B) Group-II (off fed), (C) Group-III and (D) Group-IV (zinc treated groups) also showing compact organization of stromal tissue with numerous blood vessels (bv) blood vessels and (s) stroma. H & E.

This study is based on two Experiments. In Experiment-I younger birds of 25th week of age were used and in Experiment-II comparatively older chicks of 67th weeks of age were used. In both the experiment apart from control, in three groups treatments were given in the form of off fed condition, zinc with a dosage of 25,000ppm/Kg feed and zinc with a dosage of 30,000ppm zinc/Kg feed. Zinc has been used by different workers to find out its toxic effects in pigs (Cox and Hale, 1962), rats (Magee and Spaker, 1964), cattle (Miller et al., 1965) and sheep (Ott et al., 1966). These authors reported that these animals showed sufficient tolerance to high concentration of zinc compounds. Kincaid et al (1976) investigated that the performance of broiler fowl is not adversely affected by the dietary inclusion of zinc oxide up to dosage of 1g zinc/Kg diet. However, supplementation of diets with excessive levels of zinc compounds has been suggested for the induction of a resting phase for laying hens (Shippee et al., 1979). Palafox and Ho-A (1980) demonstrated that the dietary inclusion for zinc compounds at levels providing up to 10 and 20g zinc/Kg diet caused a severe depression in food consumption, egg production and body weight. Gentle et al (1982) observed that a threshold level of ZnO addition at or above 6g Zn/Kg produced a rapid reduction in food intake of adult hens. Kincaid et al (1976) observed that dietary addition of excessive amounts of zinc compounds to diets consumed by broiler chicks showed a marked increase in liver Zn concentration and in pancreatic tissue of cockerel. Eltohamy et al (1980) used zinc oxide up to 20g/Kg and 6g Zn/Kg in two experiments respectively. They observed that food intake, body weight egg number and liver, oviduct and ovary weights/kg body weight were significantly reduced by added ZnO. They also investigated that liver, kidney and pancreatic Zn and iron concentrations were significantly elevated.

Kidd et al (1992) reported that Zn concentration in hen diets does not influence progeny performance, but Zn supplementation of broiler breeder hen diets has increased progeny livability (Virden et al., 2003). Emmert and Baker (1995) reported that sufficient body weight gains were achieved in broilers consuming only 10-60mg Zn/Kg diet. Hudson et al (2005) investigated that broiler diets supplemented with zinc improve growth rate and feed conversion, but this did not provide beneficial effects to the progeny. They suggested that broiler performance can be improved from zero to 17 days of age by zinc source supplementation.

Body Weight. In the present study, off fed condition, low (25,000ppm/Kg feed) and high zinc dosage (30,000ppm/Kg feed) treatment in Experiment-I (Batch-I where five chicks were slaughtered after 12 days) showed that there was highly significant decrease body weight because off fed condition ($P<0.001$), but no significant decrease in body weight was seen with two dosages of zinc compared to control. However, significant decrease in body weight ($P<0.001$) was seen in off fed and high zinc dosage treatment compared to low zinc dosage. In Batch-II of Experiment-I who were not slaughtered but all treatment groups were fed on normal feed up to 27^{th} day of start of experiment. Body weight (gm) highly significantly increased in off fed group (Group-II) due to feeding on normal feed. However, in Group-III low zinc dosage (25,000ppm zinc/Kg feed) and Group-IV high zinc dosage (30,000ppm zinc/Kg feed) there was no significant increase in mean body weight compared to that of control group.

Experiment-II was carried out on the same plan as was followed in Experiment-I. The only difference was that older chicks were used in this experiment. The results showed that body weight (gm) significantly decreased in Group-II, Group-II and Group-IV compared to control (Group-I) due to effect of the given treatment in Batch-I. In Batch-II of Experiment-II body weight increased significantly in Group-II (off fed) and Group-III (low zinc low) but in Group-IV (high zinc dose) there was no significant increase in body weight compared to the control (Group-I). These changes occurred due to withdrawal of treatments and provision of normal feed.

Different researchers have done work on molting using different treatments. Their studies included change during molting period and post-molting period. Aygun and Olgun (2010) have investigated the effects of non-feed and feed withdrawal on moulting and post-moulting performance in laying hens. They used different ratios of oat combined with alfalafa i.e. 100% alfalafa, 75% alfalafa and 25% of aot, 50% alfalafa and 50% oat. They observed that due to feed withdrawal during molting period there was significant body weight loss. Thirunavukkarosu et al (2007, 2009) observed body weight changes in single comb white leghorn layers at different ages (60, 65 and 70 weeks) during induced molting. They found that there was no significant difference in mean percentage of body weight loss of molt induced groups. In the post-molt period no change in body weight was observed by them. They

investigated that sixty five weeks age molt induced birds regained body weight much slower compared to other treatment groups because of high body weight loss (31.08%) during molting period. In an earlier paper Thirunavukkarosu et al (2006) also observed changes in body weight and egg production performance of induced molting white leghorn layers. They subjected the birds to feed and water restriction and seventeen hours photo period was provided. Apart from higher egg production in molting induced bird compared to controls there was no change in body weight. However, mean body weight of birds at different ages before and after induced molting showed significant difference ($P<0.01$) among the treatment groups. They observed highest percentage of body weight loss (31%) occurred in 65 weeks followed by 70 weeks (29.19%) and in 60 weeks age (28.91%) induced molt groups.

Reddy et al (2008) investigated effect of induced molting in male and female line broiler breeder hens by zinc oxide and feed withdrawal methods on post-molt performance parameters. They evaluated zinc oxide and total feed withdrawal techniques to induce molting in two breeds, i.e. Red Cornish male and white Plymouth female. They found that Cornish layers lost body weight about 16% due to zinc oxide and about 14.9% due to feed withdrawal, significantly for the corresponding figures for Rock layers were about 21.74 and 16.18% respectively. In their findings zinc oxide treatment exerted greater loss in body weight of Rock layers compared to Cornish layers. This difference in body weight loss in the two breed they suggested may be due to breed variation.

El-Gendi et al (2009) added 1% zinc to diet for 14 days for forced molting and another group for forced molting was maintained by feed restriction for 7 days. They observed that hens fed 1% zinc oxide and fasted one, showed that force molting treatments caused significant decrease in live body weight, than in the control. They investigated that in general all fast molted groups had significantly the higher rate of decrease in their live body weight when compared with their molted groups by zinc oxide supplementation. They suggested that increase in rate of body weight loss in fast molted hens may be attributed to the increasing in catabolic reactions which may have occurred due to feed withdrawal. Brake and McDaniel (1981) also found that body weight loss occurs in almost linear relationship with the length of fasted (feed withdrawal time). Loss in body weight of zinc oxide molt birds may be attributed to

the decrease in the amount of feed consumption due to unpalatability of diet supplemented with zinc. Similar results were also reported by Berry and Brake (1985) who attributed the decrease in body weight of force molted hens to negative nitrogen balance due to catabolic reaction in skeletal muscle, utilization of adipose tissue, and decrease in liver weight. The decrease in body weight in the present study two experiments carried out with restriction of food or due to addition of zinc to diet may be attributed to the reason given by Brake and Mcdaniel (1981) and Berry and Brake (1985).

Secondary Sexual Traits. In this study, the effect of the three treatments (off fed, low zinc and high zinc dosage) was also seen on secondary sexual traits (comb and wattles). Length and width of comb and wattles were measured which showed significant decrease in mean length of comb ($P<0.001$) and wattles in Group-II (off fed) and significant decrease in comb length ($P<0.001$) in Group-III (25,000ppm zinc/Kg feed) and Group-IV (30,000ppm zinc/Kg feed). Mean length and width of wattles did not show significant reduction in Group-III and Group-IV compared to control in Batch-I, Experiment-I.

Similarly, (Batch-II, Experiment-I) in the secondary sexual traits there was no significant increase in comb length and width in Group-II and Group-IV but in Group-III comb length and width showed significant increase than in control (Group-I). Wattle length and width also showed non-significant difference in the three treatment groups compared to control group. Wattle width did increase significantly in Group-III than in control group. Batch-I and Batch-II which were given the same treatments in the beginning, but later on in Batch-II treatments were withdrawn and were provided normal feed. Improvement in body weight and no significant change in length and width of comb and wattle perhaps due to normal feed provision had neutralized the effect of the three treatments (off fed, low zinc (25,000ppm zinc/Kg feed) and high zinc Group-IV (30,000ppm zinc/Kg feed) dosages.

As with Experiment-I, the effects of treatments on secondary sexual traits were also seen in Experiment-II. Comb length significantly reduced in Group-II, Group-III and Group-IV but comb width significantly reduced in Group-II and Group-IV compared to control (Group-I). Wattle length reduced significantly in off fed condition, low and high dose of zinc but wattle width significantly reduced in off fed condition and high

zinc dose compared to control. Regarding secondary sexual characters after withdrawal of treatments and provision of normal feed comb length and width increased significantly in Batch-II compared to that of Batch-I. The same effect was seen in wattle length and width.

In this study effect of treatments was investigated, but the focus of the researchers in poultry was on induced molting for improvement in egg production. They did not pay attention on changes in the secondary sexual traits no doubt they used different doses of zinc in their experiments.

Reproductive Organs. Effect of treatments due to off fed condition and with two zinc dosages was observed on ovarian weight, length and width, also on weight and length of oviduct and liver weight in the five slaughtered chicks (Batch-I, Experiment-I) Ovarian weight decreased highly significantly in Group-II and Group-IV. It was observed that off fed condition had adversely affected ovarian weight than the two zinc dosages. There was no effect on ovarian width with the three treatments. Oviductal weight and length highly significantly decreased with off fed and two dosages of zinc. Weight of the liver was adversely affected due to off fed condition and with high zinc dosage. Highly significantly reduction in liver weight was due to off fed condition and high zinc dosage.

Provision of normal feed (Batch-II, Experiment-I) has also been seen in case of ovarian weight, length and width which are not significantly different compared to controls but contrary to the results seen in the Batch-I where significant reduction in ovarian weight, length and width was observed due to the three treatments. Significant reduction in oviductal weight was seen in Group-II (off fed), Group-III (low zinc dosage) and Group-IV (high zinc dosage) compared to that of control. Also liver weight decreased significantly in the three treatment groups compared to that of control. In the case of oviductal weight and liver weight, these organs may have retained the influence of off fed condition and low and high dosage of zinc.

Reddy et al (2008) observed that the reduction in weight of the reproductive organs, i.e. ovary and oviduct in Cornish layer and Rock layers was more in feed withdrawal groups when compared to zinc oxide groups. This study also showed reduction in weight of ovary and oviduct in feed withdrawal. In both the breeds weight loss of reproductive organs was severe in feed withdrawal groups when compared to severe

stress and reduced level gonadotrophic hormones as was observed by Tanabe et al (1981) and Dickerson and Bahr (1989). Anish (2008) reported significant reduction in reproductive organs weight on molting with feed withdrawal and groups, respectively. The ovary weight decreased to 21% and 31% of initial weights in off fed and zinc treated groups on 10 day of molting. This reduction in ovary and oviduct weights indicated that there was a high level of gross reproductive regression in molted birds. Lack of gonadotropic support from the pituitary during molting has been shown to cause atresia and resorption of yolk material and ovarian regression (Berry, 2003). This could have led to reduced steroid hormone production resulting in oviduct regression.

Jackson et al (1986) are of the opinion that offering laying hens a diet containing approximately 8g Zn/Kg for at least 4 day is sufficient to cause a complete regression in the ovary and oviduct tissues. Also considerable decrease in liver fresh weight may be attributed to the depression of estrogen production and consequent inhibition of estrogen-induced lipidaemia as was reported by Griminger, 1977.

The adverse effect of the three treatments was seen on the ovarian weight, length and width, oviduct weight and length and liver weight which high significantly decreased compared to the control. Similarly, deposition of zinc in ovary, kidney and liver was significantly high in concentration due to low and high zinc dose compared to the control. In Batch-II ovarian weight was not significantly different in the three treatment groups, but ovarian length and width was significantly less than control in Group-II (off fed) and high zinc dose group (Group-IV). Similarly, oviductal length and weight were significantly less than the control in Group-II and Group-IV. However, liver weight showed no appreciable difference in the treatment groups compared to that of control.

Zinc Deposition. Zinc deposition in liver, kidney and ovary was highly significant with low and highly zinc treatment, but with off fed condition there was no deposition of zinc in liver, kidney and ovary which was expected because they were not treated with zinc. As in Batch-I, in Batch-II there was no significant zinc deposition in ovary in Group-II (off fed), but with low (Group-III) and high (Group-IV) zinc dose there was significant deposition of zinc compared to control group. Significant deposition

of zinc in kidney and liver was observed in Group-III and Group-IV compared to control.

Jackson et al (1986) investigated effects of short and long feed of zinc oxide supplemented diets on the mature female domestic fowl with special reference to tissue mineral content. They added to diet 4, 8, 12, 16 and 20gm Zn as finely powdered ZnO. They were of the view that severe depression of food intake at dietary levels of ZnO addition in excess of 1g Zn/Kg diet presumably contributed to reduction in body weight. They were not clear whether the loss in appetite was associated with high dietary levels of ZnO or may be attributed to toxic effects of the compound or to a reduction in palatability. Jackson et al (1986) observed that diets incorporating ZnO at levels 2 and 2.4g Zn/kg led to elevate Zn concentration in liver and kidney of broiler chicks.

Hormonal Levels. In this study the treatment given to chicks (off fed, low and high zinc dosages) their effects were also observed on hormonal levels like plasma estradiol , plasma progesterone and plasma corticosterone levels. In Batch-I (Experiment-I) of chicks there was highly significant reduction in levels of estradiol in the treatment groups (off fed, low and high dosages) compared to control. Similarly, there was highly significant decrease in progesterone but there was highly significant increase in corticosterone levels compared to control in Group-II, Group-III and Group-IV.

In Batch-II there was highly significant decrease of estradiol concentration in Group-II, Group-III and Group-IV compared to control, but treatment groups showed non significant differences in progesterone concentration compared to control at the end of experiment. Corticosterone concentration, like Batch-I, increased highly significantly in Group-II, III and IV compared to control.

The hormonal levels are comparable in Batch-I and Batch-II no doubt Batch-I was under stress of different treatments, but in Batch-II the treatments were withdrawn and normal feed was provided to them. Looking at the results, this may be that treatment shock, perhaps, still persists in Batch II.

Oguike et al (2005b) in their study of molt induction in layers observed that plasma progesterone levels decreased from between 0.50 and 0.60ηg/ml on day zero to undetectable levels by days 7 and 14 when they were subjected to different treatments

like (1) natural day length with water but no feed (2) natural day length with no feed no water (3) reduced day length with water but no feed (4) reduced day length with no feed. They also noticed as the number of large yellow follicles increased the concentration of progesterone in plasma increased. This study indicated that changes in ovarian weight and large yellow follicles were associated with reduction of plasma concentration of progesterone. They suggested that this phenomenon could be explained due to the absence of the large yellow yolk filled follicles in the ovaries of forced-molt groups. This could also be due to the severity of the effects of feed and water removal as well as reduction in day length on ovarian follicular hierarchy. Deprivation of feed and water could be responsible for significant decrease in plasma concentrations of progesterone to non detectable levels, since the levels gradually increased following re-feeding. They concluded that induced physiological stress resulted in regression of the ovarian activities with decrease in progesterone production. This study also showed that feed withdrawal reduced progesterone levels but on re-feeding progesterone levels did not increase perhaps treatment stress persisted.

Applying zinc oxide for molt induction may affect the ovarian function. On the other hand feed deprivation greatly decreased plasma estradiol level. This may be attributed to insufficiency of energy level available for various biological reactions concerning metabolic and hormonal coordination (El-Gendi et al., 2009). They also observed variation in estradiol and progesterone levels. The higher estradiol level was found at 4^{th} week after molt which coincided with lower level of progesterone. This may be due to negative correlation between the two ovarian hormones.

Oguike et al (2005b) investigated plasma progesterone profile and ovarian activity of forced-molt layers. They used different techniques of molt induction on commercial old layers, aged 85 weeks. The techniques they used were (1) natural day length with feed and water ad libitum (2) natural day length with water but no feed (3) natural day length with no feed no water (4) reduced day length with feed and water ad libitum (5) reduced day length with water but no feed (6) reduced day length with no feed no water designated as T_1, T_2, T_3, T_4, T_5, T_6 respectively. T_1 was control. The moult induction period was 10 days when the birds were fed low protein diet. Al-day 7, the ovaries of T_2, T_3, T_4, T_5, T_6 regressed weighing 3.43, 7.03, 5.00, 4.80g. There were

significantly ($P<0.05$) lower than the ovarian weights of 34.73 and 35.3g of T_4 and control (T_1).

Hormonal changes as a result of the treatments showed that compared to control there was highly significant reduction in estradiol levels and progesterone levels in the three treatment groups. On the other hand corticosterone levels increased highly significantly in the treatment groups compared to the control.

Changes in hormonal levels indicate that at the end of the experiment there was no significant reduction in estradiol levels, but progesterone levels highly significantly decreased in Group-II, Group-III and Group-IV compared to that of control. On the other hand plasma corticosterone levels increased highly significantly in Group-II (off fed), Group-III (low zinc dosage) and Group-IV (high zinc dosage). These results are fairly similar to these observed in Batch-I of experiment-II.

Sundaresan et al. (2007) studied cytokines in reproductive remodeling of molting White Leghorn hens. They investigated that estradiol concentrations in serum decreased significantly after 2 days of molting and reached the lowest level at 10 days of molting (35pg/ml). The same trend they also observed in progesterone levels. Progesterone concentrations in control and molting birds were 522±24 and 477±21 pg/ml respectively on the 3 days of molting. The lowest levels of estrogen and progesterone they observed in the study were 35 and 98 pg/ml respectively at the 10 of molting days. The serum corticosterone concentration reached its peak (27.4ng/ml) at the 4 day of molting and remained at the same level throughout the experimental period.

According to Elaroussi et al. (1993) plasma estradiol decreased when molting was ceased. They also observed, reproduction ceased when the estrogen Antiguans (tamoxifen) was administered to laying hens. Plasma estradiol increased with increasing estradiol (E2) dosages applied (Qin and Klandorf, 1995).

Carol et al. (2000) studied stress, corticosterone and neutrophil to lymphocyte ratio in free living Adelie Penguins. They observed the effects of fasting on corticosterone levels which increased in these birds during courtship and early incubation stage of reproduction, but only when the fast extended over 40 days. The average length of this fast for Adelie Penguins on Torgersen island is 37 days for males and 22 days for females (Burch and Vleck, 1998).During these fasts, birds progressively lost body

mass and became dehydrated as indicated by increases in hematocrit and hemoglobin concentration. Lengthy fasts are a normal part of biology of penguins. In fasting King (*Aptenodytes patogonicus*) and Magellanic (*Spheniscus magellanicus*) Penguins, corticisterone levels do not increase until the birds have early depleted fat stores and begun using protein as a primary energy store, which mat take months in large species (Cherel et al., 1988a, 1988b; Hood et al. 1998). In contrast, corticosterone levels rise through long incubation shifts (11-15 days) in Grey-headed and Black-browed Albatrosses that do not have physiological capacity to fast for as long as penguins do (Hector and Harvey, 1986).

Ovarian Follicles. In Batch-I Experiment-I Morphometric studies have shown that the three treatments (off fed, low and high zinc dosages) have affected adversely ovarian follicles. Ovarian yolky follicular diameter significantly reduced. Particularly in the case of smallest category (1-5mm) of yolky follicles in treatment groups (Group-II, Group-III and Group-IV). A similar effect was also seen on ovarian follicular diameter, ovarian oocyte diameter and follicular wall thickness. Off fed condition, low zinc dose and high zinc dose (30000ppm zinc/Kg) has affected the the development of larger follicles, i.e. follicle categories 15.1-20mm, 20.1-25mm and 25.1-30mm did not develop. In present study decrease in plasma progesterone level was observed in off fed as well as zinc treated groups. This could be correlated with the absence of large yolky follicles. Similar results have also been observed by other researchers who indicated that changes in ovarian weight and large yellow follicles are associated with the reduction of plasma concentration of progesterone (Oguike et al., 2005b; Jacquet et al., 1993; Decuypere and Verheyen, 1986).

In Batch-II of Experiment-I normal diet was provided and the three treatments were withdrawn. As a result the follicles developed in categories 15.1-20mm, 20.1-25mm and 25.1-30mm which did not develop during treatment period. Other development was that there was no significant difference in ovarian yolky follicular diameter and follicular diameter in the case of Group-II, Group-III and Group-IV compared to the control group (Group-I). On the other hand oocyte diameter and follicular wall thickness showed that in Group-II, Group-III and Group-IV there was highly significant reduction in oocyte diameter and follicular wall thickness compared to

control (Group-I). It appears that the influence of the three treatments persisted even after withdrawing the treatment and resumption of normal feed.

Oguike et al (2005a) observed in their study regarding progesterone profile and ovarian activity that the number of large yellow follicles under the experimental conditions (1) natural day length with water but no feed (2) natural day length with no feed no water (3) reduced day length with water but no feed (4) reduced day length with no feed no water decreased from 3.33 on day zero to 0.00 on day 7. By day 21 the large yellow follicles under all the four experimental conditions started regenerating, ranging between 2.33 and 3.00 and by day 49 were significantly higher than those yellow follicles which developed under reduced day light length with feed and water

In Batch-II morphometric study shows that number of ovarian yolky follicles was significantly low in Group-II compared to control in their smallest category (1-5mm). In all this categories of treatment groups there was no significant difference in mean number of yolky follicles than in control. A few yolky follicles appeared in categories 15.1-20mm, 20.1-25mm, 25.1-30mm and 30.1-35mm in Group-II and Group-IV. There was no difference in yolky follicular diameter in all the three treatment groups compared to control. Possibly this could be due to withdrawal of treatment and provision of normal feed. Similarly, follicular diameter also showed no appreciable difference in follicular diameter in the treatment groups compared to control. Oocyte diameter was highly significantly less in Group-II in category <200µm than in control (Group-I), but in other treatment group no significant change was observed. Thickness of follicular wall reduced highly significantly in categories 1-20µm and 21-40µm as well as in 81-100µm and >101µm in Group-II, Group-III and Group-IV compared to control (Group-I).

As with follicular diameter, follicular wall thickness these reduced highly significantly during the treatment period both in Experiment-I and Experiment-II but improvement in them was observed with the provision of normal food. Similarly, in the case of mean number of follicles they also reduced highly significantly in off fed condition and high zinc dose compared to control in Experiment-I and Experiment-II. It was observed that withdrawal of food had severely affected the number of follicles compared to low and high dose of zinc treatment. When food was provided mean

number of ovarian follicles increased in all the treatment groups significantly compared to those during treatment periods in both the experiments.

According to Verheyen et al (1987) when hens are subjected to forced molt, the normal size hierarchy and development of ovarian follicles is disturbed, depending upon the method used. Cessation of egg production in fasting hens is accompanied by an increase in the number of small follicles and atrophy of large yolky follicles. Regression of ovary was complete and follicles with a diameter of 9mm or more, being the main source of progesterone, were absent. Reformation of the follicular size hierarchy started after resumption of feeding a laying mash. The follicle distribution of hens fed a high zinc diet was comparable to that of fasting hens, but atrophy of yolky follicles (> 4mm) occurred earlier and reappearance of yolky follicles occurred later than in fasted hens, considering the lower number of yolky follicles (>9mm) in the hens fed a high-zinc diet compared to fasted hens. Fasting laying hens causes an immediate atrophy of the maturing ovarian follicles followed by interruption of egg production (Brake et al., 1979; Verheyen, 1986). Follicular atrophy is followed by a sharp decrease in plasma progesterone which is mainly produced by granulosa cells of the 3 largest follicles (Etches, 1984; Wells et al, 1985). Feeding a high zinc diet appeared to be a more severe treatment than fasting. The hens fed a high-zinc diet maintained minimal progesterone values and they did not regain the capacity to respond to an LH injection. These conditions are correlated with delayed redevelopment of yolky follicles for the zinc-treated hens (Verheyen et al., 1983).

In this study it was observed that large follicles did not develop during treatment period in off fed condition and in the case of high zinc dosage, also, regression of ovary occurred. During this period progesterone levels also decreased which may be correlated to follicular atrophy as has been indicated by Etches (1984) and Wells et al. (1986). Resumption of feeding and removal of zinc treatments had restored the development of large follicles and increase in production of progesterone, estradiol and decrease in the levels of corticosterone.

Molting. In Experiment-I due to the three treatments cessation of egg production started on 12^{th} day. Average egg production compared to the controls was very low. In the control, off fed condition, low zinc dosage and high zinc dosage average egg production per hen was 6.9, 3.9, 4.2 and 2.7 respectively. After the cessation of egg

production on 12th day, the egg production started but this was very slow in off fed condition which started egg production after ten days of withdrawal period. In the case of low zinc dosage and high zinc dosage the egg production had started but it was quite slow. During post-cessation or withdrawal period of egg production the average number of egg produced per hen were 11.4, 2.4, 5.0 and 5.60 in control, off fed, low zinc dosage and high zinc dosage respectively.

In experiment-II egg production of all group was also recorded during molting period which lasted for 12 days in off fed, low zinc dosage and high zinc dosage. During molting period egg production was 21.0, 4.80, 4.20 and 5.60 in control, off fed, low zinc dosage and high zinc dosage respectively. In the post molting period, as in experiment-I, in off fed condition egg production was very slow which was started after 7 days of resumption to normal feed. In the low zinc dosage egg production commenced earlier than the high zinc dosage treatment which also started after 7 days of withdrawal period. During post-molting period average egg production 11.60, 3.40, 9.20 and 5.20 in control, off fed condition, low zinc dosage and high zinc.

El-Gendi et al. (2009) observed that no-molted hens had significantly the lowest rates of egg production. On the other hand, after molting egg production sharply increased with different rates between all force molted treatments. Hens fed 1% zinc oxide and injected with distilled water increased the rate of egg production when compared with those fasted ones. This seems that applying zinc oxide as a method of molt induction had better effect on the rate of egg production when compared to fasting method.

In this study, non-molted chicks had higher rates of egg production than in the treated groups, but after molting there was gradual increase in egg production no doubt in zinc treated groups percentage of egg production was higher than in fasted group.

Histomorphology. Compared to normal group, in the treatment groups atretic follicles and disintegrated follicular and granulosa walls were observed. Deshaped nucleus, disruption of cytoplasm and loose stromal tissue and interstitial spaces were also seen. In treatment groups atretic follicles showed thick accumulation of yolky vacuoles and atrophied granulosa cells. There was also loosing of follicular epithelium, decreased percentage of thecal observed in all treatment groups. After provision of feed in the three treatment groups the ovarian follicles increased in

number particularly follicles of large size developed which had not developed during the treatment period. Re-feeding also resulted improvement in structure of nucleus and nuclear matrix. Granulosa layer with basal membrane was comparable to that of the control group. Due to re-feeding follicular epithelium was distinguishable into theca interna and theca externa. Also, thecal glands had appeared.

Meager information is available regarding the histomorphology of ovary in off fed and zinc treated groups. Present study clearly indicated that the number of non yolky small follicle is decreased in treated groups. This may be due to the effect of these treatments on pituitary ovarian axis or hypothalamic pituitary ovarian axis which might had affected the gonadotropins release with ultimate decrease in follicle number and growth accompanied by decrease in estrogen and progesterone concentration. Moreover, these treatments affected the theca and granulosa as these layers were disrupted in some of the follicles and disintegration was greater in off fed and high zinc treated groups. however, follicular atresia was observed on 6 day of molting in both treatment groups but the highest number of atretic follicles was found in the zinc treated group. Histologically the regression of ovarian stroma in the feed withdrawal group was greater than zinc treated group (Anish et al., 2008). Similar histological changes were also observed in current study but greater number of atretic follicles were noticed in off fed group compared to zinc treated groups

Sundaresan et al. (2008a) studied postovulatory follicle regression of domestic chicken (*gallus domesticus*) . In the histological changes they observed that granulosa cells rapidly lost after ovulation followed by the loss of thecal cells at a slower rate. The granulosa layer denuded from the theca layer and complexly regressed. The theca layer exhibited vacuolar degenerative changes. They also noticed increased vascularization.

CONCLUSION

In this study treatments effects due to feed withdrawal and low and high zinc dosage in young and older birds was investigated. Both, feed withdrawal and zinc dosages were effective in the reduction of body weight and secondary sexual traits. They led to regression of ovary, adversely affected oviduct weight and length. High and low zinc doses led to significant deposition of zinc in ovary, liver and kidney. These three treatment significantly reduced the levels of progesterone, estradiol and increase in corticosterone levels which ultimately disturb the reproductive process.

The number, diameter of ovarian yolky follicles, ovarian follicles oocytes significantly reduced due to low and high zinc dosages. These treatments had adversely affected the histology of ovary in which disintegration and disruption of granulosa layer, absence of large follicles and reduction in the number of small follicles. These histological changes were correlated with the hormonal disturbance.

When these treatments, were withdrawn improvement in body weight, hormonal levels, follicular diameter and number as well as restoration of histological changes were observed. Due to the appearance of large follicles, and increase in the number of small follicles. Progesterone and estradiol levels increased and corticosterone levels decreased. This study shows that feed withdrawal and zinc dosages produced stress that resulted in the drastic changes in different aspects. The changes induced by different doses of zinc were reversible so zinc can be used for molting purpose in commercial laying birds as alternative method for feed and water withdrawal techniques which are unethical.

- Adams JL (1955). Progesterone-induced unseasonable molt in Single Comb White Leghorn pullets. Poult. Sci. 34: 702-707.
- Adams JL (1956). A comparison of different methods of progesterone administration to the fowl in affecting egg production and molt. Poult. Sci. 35: 323-326.
- Ahmed AMH, Rodriguenz-Navarro AB, Vidal ML, Goutron J, Garcia-Ruiz JM and Nys Y (2005). Changes in the egg shell properties, crystallographic texture and in matrix proteins induced by molt in hens. Br. Poult. Sci. 46: 268-279.
- Akram M, Zia-ur-Rahman, Na CS; Kim SH and Ryu KS (2002). Effect of induced molting on the relative weights and hormone levels of thyroid, ovary and adrenal glands in spent laying hens. Korean J. Poult. Sci. 29: 243-247.
- Al-Batshan HA, Scheideler SE, Black BL, Garlich JD and Anderson KE (1994). Duodenal calcium uptake, femur ash and egg shell quality decline with age and increase following molt. Poult. Sci. 73: 1590-1596.
- Alodan MA and Mashaly MM (1999). Effect of induced molting in laying hens on production and immune parameters. Poult. Sci. 78: 171–177.
- Anish D, Sastry KVH, Sundaresan NR, Saxena VK, Singh R and Mohan J (2008). Reproductive tissue regression: Involvement of caspases, inducible nitric oxide synthase and nitric oxide during molting in White Leghorn hens. Anim. Reprod. Sci. 104: 329-343.
- Anjum AD (1997). "Poultry Diseases", 2nd Ed., Vet. Agr. Publication, Faisalabad, Pakistan. pp. 4-11.
- Ankney CD and Macinnes CD (1978). Nutrient reserves and reproductive performance of female lesser snow geese. Auk. J. 95: 459-471.
- Anonymous (1988). Health and Environmental Effects Chapter (6) pp. 77-81
- Anonymous (2010). Induced Molting of Layer Chickens: Welfare Implications of American Veterinary Medical Association's Animal Welfare Division.
- Anonymous (2011). Zinc. Dietary Supplement Fact Sheet. Office of dietary supplements, National Institute of Health. http://ods.od.nih.gov/factsheets/Zinc-HealthProfessional

- Armstrong DG (1985). Changes in aromatase activity in small ovarian follicles of the domestic fowl (*Gallus domesticus*) during growth and atresia. J. Endocrinol. 105: 297-301.
- Arrington LR, Santa Cruz RA, Harms RH and Wilson HR (1967). Effects of excess dietary iodine upon pullets and laying hens. J. Nutr. 92: 325-330.
- Asem EK, Molnar M and Hertelendy F (1987). Luteinizing hormone-induced intracellular calcium mobilization in granulosa cells: Comparison with forskolin and 8-bromoadenosine 3′,5′-monophosphate. Endocrinology 120: 853-859.
- Aygun A and Olgun O (2010). The effect of nonfeed and feed withdrawal molting methods on molt and postmolt performance in laying hens. Trends Anim. Vet. Sci. J. 1(2): 45-48.
- Bahr J, Gardner R, Schenck P and Shahabi N (1980). Follicular steroidogenesis: Effect of reproductive condition. Biol. Reprod. 22: 817-826.
- Baker M (1981). The relationship between adipose accumulation and reproductive dysfunction in *Gallus domesticus*. Ph.D. Dissertation. Auburn University, Auburn, AL. USA.
- Baker M, Brake J and McDaniel GR (1981). The relationship between body weight loss during a forced molt and post molt reproduction performance of caged layer. Poult. Sci. 60: 1594.
- Baker M, Brake J and McDaniel GR (1983). The relationship between body weight loss during an induced molt and post-molt egg production, egg weight, and shell quality in caged layers. Poult. Sci. 62: 409-413.
- Baker M, Brake J and Krista LM (1981). Total body lipid and uterine lipid changes during a forced molt of caged layers. Poult. Sci. 60: 1593.
- Bar A, Razaphkovsky V, Wax E and Malka Y (2001). Effect of age at molting on post-molting performance. Poult. Sci. 80: 874-878.
- Bartlett JMS, Weinbauer GF and Nieschlag E (1990). Stability of spermatogenic synchronization achieved by depletion and restoration of vitamin A in rats. Biol. Reprod. 42: 603-612.

- Bartlett JR and Smith MO (2003). Effects of different levels of Zn on the performance and immunocompetence of broilers under heat stress. Poult. Sci. 82: 1580-1588.
- Batal AB, Parr TM and Baker DH (2001). Zinc bioavailability in tetrabasic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. Poult. Sci. 80: 87-90.
- Battley PF, Piersma T, Dietz MW, Tang S, Dekinga A and Hulsman K (2000). Empirical evidence for differential organ reductions during trans-oceanic bird flight. Proc. Royal Soc. 267: 191-195.
- Bell DD (2001). Economic implications of controversial layer management programs. In: Proceedings of the 50th Western Poultry Disease Conference, Davis, CA. pp. 83-91.
- Bell DD (2002). Commercial chicken Meat and Egg production. 5th ed. Kluwer Academic Publishers, MA, USA, pp. 1059-1077.
- Bell DD (2003). Historical and current molting practices in the U.S. table egg industry. Poult. Sci. 82: 965-970.
- Bell DD, Chase B, Douglass A, Hester P, Mench J, Newberry R, Shea-Moore M, Stanker L, Swanson J and Armstrong J (2004). UEP uses scientific approach in its establishment of welfare guidelines. Feedstuffs 76: 1-9.
- Bell DD, Swanson MH and Johnston G (1976). A comparison of force molting methods. Progress in Poultry. University of California Cooperative Extension, Davis, CA. USA.
- Benoit J (1962). Hypothalamo-hypophyseal control of the sexual activity in birds. Gen. Comp. Endocrinol. 1: 254-274.
- Ben Nathan D, Heller ED and Perek M (1977). The effect of starvation on antibody reduction of chicks. Poult. Sci. 56: 1468-1471.
- Berger LL (2003)**.** Zinc: Nutritional and Pharmacological Roles. University of Illinois. www.thepoultrysite.com.
- Berry WD (2003). The physiology of induced molting. Poult. Sci. 82: 971-980.

- Berry WD and Brake J (1985). Comparison of parameters associated with molt induced by fasting, zinc, and low dietary sodium in caged layers. Poult. Sci. 64: 2027-2036.
- Berry WD and Brake J (1987). Post-molt performance of laying hens molted by high dietary zinc, low dietary sodium and fasting: egg production and egg shell quality. Poult. Sci. 66: 218-226.
- Berry WD and Brake J (1991). Research note: Induced molt increases egg shell quality and Calbindin- D28k content of egg shell gland duodenum of aging hens. Poult. Sci.70: 655-657.
- Berzin NI and Bauman VK (1987). Vitamin-A-dependent zinc-binding protein and intestinal absorption of Zn in chicks. Br. J. Nutr. 57: 255-268.
- Beuving G and Vonder GMA (1978). Effect of stressing factors on corticosterone levels in the plasma of laying hens. Gen. Comp. Endocrinol. 35: 153-159.
- Beuving G and Vonder GMA (1986). Comparison of the adrenal sensitivity to ACTH of laying hens with immobilization and serum baseline levels of corticosterone. Gen. Comp. Endocrinol. 62: 353-358.
- Biggs PE, Douglas MW, Koelkebeck KW and Parsons CM (2003). Evaluation of non-feed removal methods for molting programs. Poult. Sci. 82: 749-753.
- Biggs PE, Persia ME, Koelkebeck KW and Parsons CM (2004). Further evaluation of non-feed removal methods for molting programs. Poult. Sci. 83: 745-752.
- Blalock TL and Hill CH (1988). Studies on the role of iron in zinc toxicity in chicks. Biol. Trace Elem. Res. 17: 17-29.
- Bradley EL and Holmes WN (1971). The effects of hypophysectomy on adrenocortical function in the duck (*Anas platyrhynchos*). J. Endocrinol. 49: 437-457.
- Brake J (1992). Mechanisms of and metabolic requirements for complete and rapid reproductive rejuvenation during an induced molt-A brief review. Ornis Scandinavica 23: 335-339.
- Brake J (1993). Recent advances in induced molting. Poult. Sci. 72: 929-931.

- Brake J and Mc-Daniel GR (1981). Factors affecting broiler breeder performance. 3. Relationship of body weight during fasting to post-molt performance. Poult. Sci. 60: 726-729.
- Brake J and Thaxton P (1979a). Physiological changes in caged layers during a forced molt. 2. Gross changes in organs. Poult. Sci. 58: 707-716.
- Brake J and Thaxton P (1979b). Physiological changes in caged layers during a forced-molt. 1. Body temperature and selected blood constituents. Poult. Sci. 58: 699-706.
- Brake J, Baker M and Mannix JG (1981b). Weight loss characteristics of the body, liver, ovary, oviduct, and uterine lipid during a forced molt and their relationship to performance. Poult. Sci. 60: 1628 (abstr).
- Brake J, Baker M, Morgan GW and Thaxton P (1982). Physiological changes in caged layers during a forcedmolt. 4. Leucocytes and packed cell volume. Poult. Sci. 61: 790-795.
- Brake J, Berry WD and Thaxton P (1985). Cellular changes in the spleen during an induced molt. Poult. Sci. 64: 1031-1034.
- Brake J, Morgan GW and Thaxton P (1981a). Recrudescence of the thymus and repopulation of lymhocytes during an artificially induced molt in the domestic chicken: Proposed model system. Dev. Comp. Immunol. 5: 105-112.
- Brake J, Thaxton P and Benton EH (1979). Physiological changes in caged layers during a forced molt. Plasma thyroxine, plasma tri-iodothyronine, asrenal cholesterol and total adrenal steroids. Poult. Sci. 58: 1345-1350.
- Braw-Tal R, Yossefi S, Pen S, Shinder D and Bar A (2004). Hormonal changes associated with ageing and induced molting of domestic hens. Br Poult. Sci. 45: 815-822.
- Breeding SW, Brake J, Garlich JD and Johnson AL (1992). Molt induced by dietary zinc in a low-calcium diet. Poult. Sci. 71: 168-180.
- Breitschwerdt EB, Armstrong PJ and Robinette CL (1986). Three cases of acute zinc toxicosis in dogs. Vet. Hum. Toxicol 28: 109-117.
- Bucher TL and Vleck CM (1998). Patterns of nest attendance and relief in Adelie Penguins (*Pygoscelis adeliae)*. Antarctic J. USA. 32: 103-104.

- Burrell AL, Dozier WA, Davis AJ, Compton MM, Freeman ME, Vendrell PF and Ward TL (2004). Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. Br. Poult. Sci. 45: 225-263.
- Burton M and Burton R (1980). The New International Wildlife Encyclopedia. N.C.L.C. Limited, London, UK.
- Butler PJ and Woakes AJ (1990). The physiology of bird flight. In: Bird Migration, ed. (E. Gwinner) Springer. Berlin, UK. pp. 300-318.
- Callard IP, Lance V, Salhanick AR and Barad D (1978). The annual ovarian cycle of chrysemys picta: Correlated charges in plasma steroid and parameters of vitellogenosis. Gen. Comp. Endocrinol. 35: 245-257.
- Campol JL and Da´vila SG (2002). Effect of Photoperiod on heterophil to lymphocyte ratio and tonic immobility duration of chickens. Poult. Sci. 81: 1637-1639.
- Cantor AH and Johnson TH (1984). Inducing pauses in egg production of Japanese quail with dietary zinc. Poult. Sci. 63: 10.
- Carnevali O, Mosconi G, Angelini F, Limatola E, Ciarcia G and Polzonetti Magni A (1991). Plasma vitellogenin and 17B estradiol levels during the reproductive cycle of Podarcis s. sicular Raf. Gen. Comp. Endocrinol. 84(3): 337-343.
- Carol MV, Vertalino N, Vleck D and Bucher TL (2000). Stress, corticosterone, and heterophil to lymphocyte ratios in free-living adelie penguins. The Condor. 102: 392-400.
- Carsia RV and Harvey S (2000). Reproductive parameters and plasma corticosterone concentrations. Sturkies Avian Physiology. G. Causey Wittow, ed. 5th ed. Academic Press, San Diego, USA. pp. 511-512.
- Case CL and Carlson MS (2002). Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J. Anim. Sci. 80: 19-17.
- Chandra RK (1984) Excessive intake of zinc impairs immune responses. J. Am. Med. Assoc. 252: 1443-1446.
- Cherel Y, Leloup J and Le Maho Y (1988a). Fasting in king penguin II. Hormonal and metabolic changes during molt. Am. J. Physiol. 254: 178-184.

- Cherel Y, Robin JP, Walch O, Karmann H, Netchitailo P and Le Maho Y (1988b). Fasting in king penguin I. Hormonal and metabolic changes during breeding. Am. J. Physiol. 254: 170-177.
- Chowdhury VS, Noshibori M and Yoshimura Y (2004). Changes of the mRNA expression of TGF*â* receptor types II and III in the interior pituitary during induced molting in hens. J. Poult. Sci. 41: 140-146.
- Christian P and West KP (1998). Interactions between zinc and vitamin A: An update. Am. J. Nutr. 68: 435-441.
- Christmas RB, Harms RH and Junqueira OM (1985). Performance of Single Comb White Leghorn subjected to 4 or 10-day feed withdrawal force rest procedures. Poult. Sci. 64: 2321-2324.
- Cox DH and Hale OM. (1962). Liver iron depletion without copper loss in swine for excess zinc. J. Nutr. 77: 225-228.
- Craig JV and Craig JA (1985). Corticosteroid levels in White Leghorn hens as affected by handling, laying-house environment, and genetic stock. Poult. Sci. 64: 809-816.
- Creger CR and Scott JT (1977). Dietary zinc as an effective resting agent for the laying hen. Poult. Sci. 56: 1706 (Abstr).
- Cunningham DL and McCormick CC (1985). A multicycle comparison of dietary zinc and feed removal, molting procedures, production and income performance. Poult. Sci. 64: 253-260.
- Daugherty SB (2002). Effects of prenatal energy supplementation and prenatal prebreeding trace mineral/vitamin E supplemental on calf health and reproductive performance in beef cows. M. S. Thesis. Texas A&M University, College Station, TX. USA.
- Davis AJ, Lordelo MM and Dale N (2002). Use of cottonseed meats in molting programs. J. Appl. Poult. Res. 11: 175-178.
- Davis MS (1998). Effects of supplemental copper on cellular and humeral immune response and performance of newly weaned calves. MS. Thesis. Texas A&M University, College Station, TX. USA.
- Davis GS and Siopes TD (1985). Adrenal cortical response of tom poults. Poult. Sci. 64(11): 2189-2194.

- Davis GS, Anderson KE and Carrol AS (2000). The effects of long term caging and molt of Single Comb White Leghorn hens on heterophil to lymphocyte ratios, corticosterone and thyroid hormones. Poult. Sci. 79: 514–518.
- Dean CE, Hargis BM and Hargis PS (1991). Effects of zinc toxicity on thyroid function and histology in broiler chicks. Toxicol. Lett. 57: 309-318.
- Decuypere E and Verheyen G (1986). Physiological basis of induced molting and tissue regeneration in fowls. World's Poult. Sci. J. 42: 56-68.
- Decuypere E, Leenstra F, Huybrechts LM, Feng PY, Arnonts S, Herremans M and Nys M (1993). Selection for weight gain or food conversion in broiler affects the progesterone production capacity of large follicles in reproductive adult breeders. Br. Poult. Sci. J. 34: 543-552.
- Dehnhard M, Schreer A, Krone O, Jewgenow K, Krause M and Grossmannc R (2003). Measurement of plasma corticosterone and fecal glucocorticoid metabolites in the chicken (Gallus domesticus), the great cormorant (Phalacrocorax carbo), and the goshawk (*Accipiter gentilis*). Gen. Comp. Endocrinol. 131: 345-352.
- Dewar WA, Wight PAL, Pearson RA and Gentle MJ (1983). Toxic effects of high concentrations of zinc oxide in the diet of the chick and laying hens. Br. Poult. Sci. 24: 397-404.
- Dickerman RW, Wise TH and Bahr JM (1992). Effect of ovarian regression and molt on plasma concentrations of thymosin beta 4 in domestic hens (*Gallus domesticus*). Domest. Anim. Endocrinol. 9: 297-304.
- Dickerman RW and Bahr JM (1989). Molt induced by gonadotropin-releasing hormone agonist as a model for studying endocrine mechanisms of molting in laying hens. Poult. Sci. 68(10):1402-1408
- Donalson LM, Kim WK, Woodward CL, Herrera P, Kubena LF, Nisbet DJ and Ricke SC (2005). Utilizing different ratios of alfalfa and layer ration for molt induction and performance in commercial laying hens. Poult. Sci. 84: 362-369.

- Douglas CR, Harms RH and Wilson HR (1972). The use of extremely low dietary calcium to alter the production pattern of laying hens. Poult. Sci. 51: 2015-2020.
- Droual R, Meteyer CU and Galey FD (1991). Zinc toxicosis due to ingestion of a penny in a gray-headed chachalaca (Ortalis cinereiceps). Avian Dis. 35: 1007-1011.
- Dunkley CS, Friend TH, McReynolds JL, Kim WK, Dunkley KD, Kubena LF, Nisbet DJ and Ricke SC (2008a). Behavior of laying hens on alfalfa crumble molt diets. Poult. Sci. 87: 815-822.
- Dunkley CS, Friend TH, McReynolds JL, Woodward CL, Kim WK, Dunkley KD, Kubena LF, Nisbet DJ and Ricke SC (2008b). Behavioral responses of laying hens to different alfalfa-layer ration combinations fed during molting. Poult. Sci. 87: 1005-1011.
- Dunkley CS, McReynolds JL, Dunkley KD, Kubena LF, Nisbet DJ and Ricke SC (2007). Molting in Salmonella Enteritidis-Challenged Laying Hens Fed Alfalfa Crumbles. III. Blood Plasma Metabolite Response. Poult. Sci. 86: 2492-2501.
- Durant JA, Corrier DE, Byrd JA, Stanker LH and Ricke SC (1999). Feed deprivation affects crop environment and modulates *Salmonella enteritidis* colonization and invasion of leghorn hens. Appl. Environ. Microbiol. 65: 1919-1923.
- Ebru Odabaşilar E (2006). Forced molting methods in laying hens. J. Fac. Vet. Med. Univ. Erciyes. 3(2): 111-115.
- El Deek AA and AI-Harthi MA (2004). Post molt performance parameters of broiler breeder hens associated with molt induced feed restriction, high dietary zinc, and fasting. Int. J. Poult. Sci. 3(7): 456-462.
- Elaroussi MA, Forte LR, Egber SL and Biellier HV (1993). Adaptation of the kidney during reproduction: Role of estrogen in the regulation of responsiveness to parathyroid hormone. Poult. Sci. 72: 1548-1556.
- El-Gendi GM, Samak HR and Mohamed AA (2009). Effect of induced molting on some productive and physiological traits in Hy-line hens. Egypt. Poult. Sci. 29(I): 385-405.

- Eltohamy MM, Takahara H and Okamato M (1980). Effects of zinc on intact and castrated cockerels. Br. Poult. Sci. 21: 363-369.
- Emmert JL and Baker DH (1995). Zinc stores in chickens delay the onset of zinc deficiency symptoms. Poult. Sci. (74): 1011-1021.
- Ensminger ME, Oldfield JE and Heinemann WW (1990). Feeds and Nutrition. The Ensminger Publishing Company, Clovis, CA. pp. 8-110.
- Ernst RA (1998). Lighting Programs for table Egg Layers Cooperative Extension University of California, Department of Animal Science, University of California, Davis, CA 95616. USA.
- Etches RJ (1984). Maturation of ovarian follicles. In: Reproductive Biology of Poultry. (Ed) Cunningham FJ, Lake PE and Hewitt D. Harlow, UK. pp. 51-57.
- Etches RJ (1990). The ovulatory cycle of the hen. Critical Reviews in Poult. Biol. 2: 293-319.
- Etches RJ (1993). Reproduction in poultry. In: Reproduction in domesticated Animals. (Ed) King GJ. Elsevier, Huddersfield, UK.
- Etches RJ (1996). Reproduction in Poultry. CAB International, Wallingford, UK. pp. 267-397.
- Etches RJ and Duke CE (1984). Progesterone androstenedione and oestradial content of theca and granulosa tissues of four largest ovarian follicles during ovulatory cycle of hen. J. Endocrinol. 103: 71-76.
- Etches RJ, Williams JB and Rzasa J (1983). Corticosterone and nutritionally induced ovarian regression in the hen. Poult. Sci. 62: 1417.
- Etches RJ, Williams JB and Rzasa J (1984). Effects of corticosterone and dietary changes in the hen on ovarian function, plasma LH and steroids and the response to exogenous LHRH. J. Reprod. Fertil. 70: 121-130.
- Faitarone ABG, Garcia EA, Pizzolante CC, Molino AB, Pelícia K and Berto DA (2008a). Forced-molting methods and their effects on the performance and egg quality of Japanese quails (Coturnix japonica) in the second laying. Braz. J. Poult. Sci. 10: 53-57.
- Faitarone ABG, Garcia EA, Pizzolante CC, Molino AB, Pelícia K and Berto DA (2008b). Feeding programs to induce molting in Japanese quails (Coturnix japonica). Braz. J. Poult. Sci. 10(1): 11-15.

- Forbes RM (1984). Use of laboratory animals to define physiological functions and bioavailability of zinc. Fed. Proc. 43: 2835-2839.
- Fosmire GJ (1990). Zinc toxicity. Am. J. Clin. Nutr. 51:225-227.
- Freeman BM, Manning ACC and Flack IH (1980). Short term stressor effects of food withdrawal on the immature fowl. Comp. Biochem. Physiol. 67: 569-571.
- Freeman BM, Manning ACC and. Flack IH (1981). The effects of restricted feeding on adrenal cortical activity in the immature domestic fowl. Br. Poult. Sci. 22: 295-303.
- Garfinkel D (1986). Is aging inevitable? The intracellular zinc deficiency hypothesis of aging. Med. Hypotheses. 19: 117-137.
- Garlich J, Brake J, Parkhurst CR, Thaxton JP and Morgan GW (1984). Physiological profile of caged layers during one production year, molt, and post-molt: Egg production, egg shell quality, liver, femur and blood parameters. Poult. Sci. 63: 339-343.
- Garlich J, Morris C and Brake J (1982). External bone volume, ash, and fat-free dry weight of femurs of laying hens fed diets deficient or adequate in phosphorus. Poult. Sci. 61: 1003-1006.
- Garlich JD and Parkhurst CR (1982). Increased egg production by calcium supplementation during the initial fasting period of a forced molt. Poult. Sci. 61: 955-961.
- Gast RK and Ricke SC (2003). Current and future prospects for induced molting in laying hens. Poult. Sci. 82: 964.
- Gentle MJ, Dewar WA, Wight PAL and Dick KM (1982). The effects of high dietary zinc on food intake in the domestic fowl. J. Intake Res. 3(1): 53-60.
- Giampauli J, Pedroso AA and Moraes VMB. (2005). Desempenho e qualidade de ovos de poedeiras após a muda forçada suplementadas com probióticos em diferentes fases de criação.Ciência Animal Brasileira 6(3):179-186.
- Gibson SW, Stevenson MH and Jackson N (1986). Comparison of the effects of feeding diets supplemented with zinc oxide or zinc acetate on the performance and tissue mineral content of mature female fowls. Br. Poult. Sci. 27(3): 391-402.

- Gilbert AB (1971). The ovary. In The Physiology and Biochemistry of the Domestic Fowl. (Eds). Academic Press, London. Vol 3: pp. 1163-1208.
- Gilbert AB and Wells JW (1984). Structure and function of the ovary. In Reproductive Biology of Poultry. (Eds). Ltd. Oxford. UK. pp. 15-27.
- Gilbert G and Blair R (1975). A comparison of the effects of two low-calcium diets on egg production in the domestic fowl. Br. Poult. Sci. 16: 54-62.
- Gildersleeve RP, Johnson WA, Satterlee DG and Scott TR (1979). Daily rhythms of plasma corticosterone in hens during production and before a forced molt. Poult. Sci. 58: 1061.
- Golden NJ, Marks HH, Coleman ME, Schroeder CM, Bauer Jr NE and Schlosser WD (2008). Review of induced molting by feed removal and contamination of eggs with Salmonella enterica serovar Enteritidis. Vet. Microbiol. 131: 215-228.
- Goodman BL, Norton Jr RA and Diambra OH (1986). Zinc oxide to induce molt in layers. Poult. Sci. 65: 2008-2014.
- Gracey JF and Collins DS (1992). Meat Hygiene. Balliere Tind. Lond. pp. 165-166.
- Greenman DL and Zarrow MX (1961). Steroids and carbohydrate metabolism in the domestic bird. Proc. Soc. Exp. Biol. Med. 106: 459-462.
- Griminger P (1977). Effect of dietary copper sulphate on egg production and shell thickness. Poult. Sci. 56: 359-361.
- Gross WB (1989). Factors affecting chicken thrombocyte morphology and the relationship with heterophil:lymphocyte ratios. Br. Poult. Sci. 30: 919-925.
- Gross WB and Siegel HS (1983). Evaluation of the heterophil lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27: 972-979.
- Hahn JD and Baker DH (1993). Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. J. Anim. Sci. 71: 3020-3024.
- Hammond RW, Burke WH and Hertelendy F (1981). Influence of follicular maturation on progesterone release in chicken granulosa cells in response to turkey and ovine gonadotropins. Biol. Reprod. 24: 1048-1055.
- Harms RH (1981). Effect of removing salt, sodium or chloride from the diet of commercial layers. Poult. Sci. 70: 333-336.

- Harvey S and Klandorf H (1983). Reduced adrenocortical function and increased thyroid function in fasted and refed chickens. J. Endocrinol. 98:129-135.
- Harvey S, Klandorf H and Pinchasov Y (1983). Visual and metabolic stimuli cause adrenocortical suppression in fasted chickens during refeeding. Neuroendocrinology. 37: 59-63.
- Harvey S, Merry BJ and Phillips JG (1980). Influence of stress on the secretion of corticosterone in the duck *(Anas platyrhynchos*). J. Endocrinol. 87: 161-171.
- Hassan MS (1996). Physiological changes in laying fowl during forced molting. M. Sc. Thesis, Fac. Of Agric. Cairo Univ. Egypt.
- Hector JAL and Harvey S (1986). Corticosterone secretion through long incubation shifts in Diomedea albatrosses. Gen. Comp. Endocrinol. 62: 349-352.
- Hermayer KL, Stake PE and Shippe RL (1977). Evaluation of dietary zinc, cadmium, tin, lead, bismuth and arsenic toxicity in hens. Poult. Sci. 56(Suppl. 1): 1721-1722.
- Hermes JC (2003a). Why did my chickens stop laying? A pacific northwest extension publication. Oregon State University, Washington State University, University of Idaho.
- Hermes JC (2003b). Why did my chickens stop laying? Oregon State University, Washington State University, University of Idaho. A Pacific Northwest Extension Publications (PNW) 565.
- Heryanto B, Yoshimura Y and Tamur T (1997a). Cell proliferation in the process of oviductal tissue remodeling during induced molting in hens. Poult. Sci. 76: 1580-1586.
- Heryanto B, Yoshimura Y, Tamura T and Okamoto T (1997b). Involvement of apoptosis and lysosomal hydrolase activity in the oviductal regression during induced molting in chickens: a cytochemical study for end labeling of fragmented DNA and acid phosphatase. Poult. Sci. 76: 67-72.
- Highfill C (1998). A-zinc of zinc poisoning. Winged wisdom pet bird Magzine, Ezine. http://www.birdsnways.com/wisdom/ww14eiii.htm.

- Ho SM, Kleis S, Mcpherson R, Heisermann GJ and Callard IP (1982). Regulation of vitellogenesis in reptiles. Herpetologica 38: 40-50.
- Hocking PM, Channing CE, Waddington D and Jones RB (1996). Age related changes in fear, sociality and pecking behaviours in two strains of laying hen. Br. Poult. Sci. 42: 414-423.
- Hocking PM, Maxwell MH, Mitchell MA and Robertson GW (1998). Welfare and productivity of restricted broiler breeder females fed ad libitum or restricted after the peak of egg production. Br. Poult. Sci. 39: 16.
- Holt PS (1992a). Effect of induced molting on B cell and CT and CT2 T-cell numbers in spleen and peripheral blood of White Leghorn hens. Poult. Sci. 71: 2027-2034.
- Holt PS (1992b). Effects of induced molting on immune responses of hens. Br. Poult. Sci. 33: 165-175.
- Holt PS and Porter Jr RE (1992a). Microbiological and histopathological effects of an induced-molt fasting procedure on a Salmonella enteritidis infection in chickens. Avian Dis. 36: 610-618.
- Holt PS and Porter Jr RE (1992b) Effect of induced molting on the course of infection and transmission of *Salmonella enteritidis* in White Leghorn hens of different ages. Poult. Sci. 71: 1842-1848.
- Holt PS, Buhr RJ, Cunningham DL and Porter Jr. RE (1994). Effect of two different molting procedures on a *Salmonella entertidis* infection. Poult. Sci. 73: 1267-1275.
- Hood LC, Boersmaa PD and Wingfield JC (1998). The adrenocortical response to stress in incubating Magellanic Penguins (*Spheniscusm agellunicus*). Auk. J. 115: 76-84.
- Hoshino S, Suzuki M, Kakegawa T, Imai K, Wakita M, Kobayashi Y and Yamada Y (1988a). Changes in plasma thyroid hormones, luteinizing hormone, estradiol, progesterone and corticosterone of laying hens during a forced-molt. Comp. Biochem. Physiol. A Comp. Physiol. 90: 355-359.

- Hoshino S, Suzzuki M, Kakegawa T, Imai K, Wakita M, Kobayashi Y and Yamada Y (1988b). Changes in thyroxin and triiodothyronine during spontaneous molt in the hen, *Gallus domesticus*. Comp. Biochem. Physiol. 90: 327-332.
- Huang ESR and Nalbandov AV (1978). Estrogen produced by dissociated chicken follicular cells. Biol. Reprod. 18 (1): 39.
- Hudson BP, Dozier WA and Wilson JL (2005). Broiler live performance response to dietary zinc source and the influence of zinc supplementation in broiler breeder diets. Ani. Feed Sci. Tech. 118: 329-335.
- Hughes BO and Whitehead CC (1979). Behavioural changes associated with the feeding of low-sodium diets to laying hens. Appl. Anim. Ethol. 5: 255-266.
- Hughes BO and Wood-Gush DGM (1973). An increase in activity of domestic fowls produced by nutritional deficiency. Anim. Behav. 21: 10-17.
- Hurwitz S, Bornstein S and Lev Y (1975). Some responses of laying hens to induced arrest of egg production. Poult. Sci. 54: 415-422.
- Hurwitz S, Wax E, Nisenbaum Y, Benmoshe M and Plavinik I (1998). The response of laying hens to induced molt as affected by strain and age. Poult. Sci. 77: 22-31.
- Hussein AS (1996). Induced molting procedures in laying fowl. World's Poult. Sci. 52: 175-187.
- Hussein AS, Cantor AH and Johnson TH (1989). Comparison of the use of dietary aluminium with the use of feed restriction for force-molting laying hens. Poult. Sci. 68: 891-896.
- Hussein AS, Cantor AH and Johnson TH (1988). Use of high dietary levels of Aluminium and zinc for inducing pauses in egg production of Japanese quails. Poult. Sci. 67*:* 1157-1165.
- Imai K and Nalbandov AV (1978). Plasma and follicular steroid levels of laying hens after the administration of gonadotropins. Biol. Reprod. 19: 779-784.

- Jackson N, Gibson SW and Stevenson MH (1986). Effects of short and long-term feeding of zinc oxide-supplemented diets on the mature, female domestic fowl with special reference to tissue mineral content. Br. J. Nutr. 55: 333-349.
- Jacquet JM, Seigneurin F and De Rivers M (1993). Induced-molting in cockrels effect on sperm production, plasma concentration of luteinizing hormone, testesterone and thyroxine on pituitary sensitivity to luteinizing hormone-releasing hormone. Br. Poult. Sci. 34: 765-775.
- Johnson A and Brake J (1992). Zinc-induced molt: Evidence for a direct inhibitory effect on granulose cell steroidogenesis. Poult. Sci. 71: 161-167.
- Johnson AL and Van Tienhoven A (1980a). Hypothalamo-hypophyseal sensitivity to central injection of progesterone and LH-RH in the laying and molting hen. Adv. Physiol. Sci. 33: 99-107.
- Johnson AL and Van Tienhoven A (1980b). Plasma concentrations of six steroids and LH during the ovulatory cycle of the hen (*Gallus domesticus*). Biol. Reprod. 23: 386-393.
- Johnson PA, Dickerman RW and Bahr JM (1986). Decreased granulosa cell, luteinizing hormone sensitivity and altered thecal estradiol concentration in the aged hen (*Gallus domesticus*). Biol. Reprod. 35: 641-646.
- Joseph MM and Meier AH (1973). Daily rhythms of plasma corticosterone in the common pigeon. Gen. Comp. Endocrinol. 20: 326-330.
- Joyner CJ, Peddie MJ and Taylor TG (1987). The effect of age on egg production in the domestichen. Gen. Comp. Endocrinol. 65: 331-336.
- Kaya S, Derya H, Umucalilar Haliloglu S and Ipek H (2001a). Effect of Dietary Vitamin A and Zinc on Egg Yield and Some Blood Parameters of Laying Hens. Turk. J. Vet. Anim. Sci. 25: 763-769.
- Kaya S, Kececi T and Haliloglu S (2001b). Effects of zinc and vitamin A supplements on plasma levels of thyroid hormones, cholesterol, glucose and egg yolk cholesterol of laying hens. Res. Vet. Sci. 71: 135-139.
- Kaya S, Ortatatli M and Haliloglu S (2002). Feeding diets supplemented with zinc and vitamin A in laying hens: effects on histopathological findings and tissue mineral contents. Res. Vet. Sci. 73: 251-257.

- Kazacos EA and Van Vleet JF (1989). Sequential ultrastructural changes of the pancreas in zinc toxicosis in ducklings. Am. J. Pathol. 134: 581-595.
- Keshavarz K and Quimby FW (2002). An investigation on different molting techniques with an emphasis on animal welfare. J. Appl. Poult. Res. 11: 54-67.
- Khoshoei EA and Khajali F (2006). Alternative induced-molting methods for continuous feed withdrawal and their influence on postmolt performance of laying hens. Int. J. Poult. Sci. 3: 47-50.
- Kidd MT, Anthony NB and Lee SR (1992). Progeny performance when dams and chicks are fed supplemental zinc. Poult. Sci. 71: 1201-1206.
- Kidd MT, Ferket PR and Qureshi MA (1996). Zinc metabolism with special reference to its role in immunity. World's Poult. Sci. J. 52: 309-324.
- Kincaid RL, Miller W J, Jensen LS, Hampton DL, Neathery MW and Gentry RP (1976). Effect of high amounts of dietary zinc and age upon tissue zinc in young chicks. Poult. Sci. 55: 1954-1957.
- Koch JM, Lay DC, Macmunn KA, Moritz JS and Wilson ME (2007). Motivation of hens to obtain feed during a molt induced by feed withdrawal, wheat middlings or melengestrol acetate. Poult. Sci. 86: 614-620.
- Koelkebeck KW (2001). Molting, bird density and animal welfare. Illini poulty net. University of Illinois Extension. USA.
- Koelkebeck KW and Anderson KE (2007). Molting layers alternative methods and their effectiveness. Poult. Sci. 86: 1260-1264.
- Koelkebeck KW, Parsons CM, Biggs P and Utterback P (2006). Nonwithdrawal molting programs. J. Appl. Poult. Res. 15: 483-491.
- Kubena LF, Byrd JA, Moore RW, Ricke SC and Nisbet DJ (2005). Effects of drinking water treatment on susceptibility of laying hens to Salmonella enteritidis during forced molt. Poult. Sci. 84: 204-211.
- Kuenzel WJ (2003). Neurobiology of molt in avian species. Poult. Sci. 82: 981-991.
- Kwon YM, Kubena LF, Woodward CL, Byrd JA, Moore RW, Nisbet DI and Ricke SC (2001). Use of an alfalfa diet for molting in Leghorn hens to reduce Salmonella enteritidis colonization and invasion. Poult. Sci. 80(Suppl. I): 90 (Abstr).

- Lance VA (1989). Reproductive cycle of the American alligator. Am. Zool. 29: 999-1018.
- Landers KL, Howard ZR, Woodward CL, Birkhold SG and Ricke SC (2005b). Potential of alfalfa as an alternative molt induction diet for laying hens: Egg quality and consumer acceptability. Bioresour. Technol. 96: 907-911.
- Landers KL, Moore RW, Dunkley CS, Herrera P, Kim WK, Landers DA, Howard ZR, McReynolds JL, Byrd JA, Kubena LF, Nisbet DJ and Ricke SC (2008a). Organ weight and serum triglyceride responses of older (80week) commercial laying hens fed an alfalfa meal molt diet. Bioresour. Technol. 99: 6692-6696.
- Landers KL, Moore RW, Dunkley CS, Herrera P, Kim WK, Landers DA, Howard ZR, McReynolds JL, Byrd JA, Kubena LF, Nisbet DJ and Ricke SC (2008b). Immunological cell and serum metabolite response of 60-week-old commercial laying hens to an alfalfa meal molt diet. Bioresour. Technol. 99: 604-608.
- Landers KL, Woodward CL, Li X, Kubena LF, Nisbet DJ and Ricke SC (2005 a). Alfalfa as a single dietary source for molt induction in laying hens. Bioresour. Technol. 96: 565-570.
- Lantzsch HJ and Schenkel H (1978). Effect of specific nutrient toxicities in animals and man: Zinc. CRC handbook series in nutrition and food; section E-nutritional disorders. Effect of nutrient excesses and toxicities in animal and man. M. Recheigl, ed. CRC Press Inc., West Palm Beach, FL. USA. Vol. 1. pp. 291-307.
- Le Maho Y (1977). The emperor penguin: A strategy to live and breed in the cold. Am. Sci. J. 65: 680-693.
- Lee K (1982). Effect of forced molt period on postmolt performance of Leghorn hens. Poult. Sci. 61: 1594-1598.
- Leska A and Dusza L (2007). Seasonal changes in the hypothalamopituitary-gonadal axis in birds. Reprod. Biol. 7(2): 99-126.
- Lien RJ and Siopes TD (1989). Turkey plasma thyroid hormone and prolactin concentrations throughout an egg laying cycle and in relation to photorefractoriness. Poult. Sci. 68: 1409-1417.

- Lu J and Combs Jr GF (1988). Effect of excess dietary zinc on pancreatic exocrine function in the chick. J. Nutr. 118: 681-689.
- Luck MR and Scanes CG (1980). Ionic and endocrine factors influencing the secretion of luteinizing hormone by chicken anterior pituitary cells *in vitro*. Gen. Comp. Endocrinol. 41: 260-265.
- Magee AC and Spaker S (1964). Effects of dietary supplements on young rats fed high levels of zinc. J. Nutr. 82: 209-216.
- Maho Le Y, Karmann H, Briot D, Handrich Y, Robin JP, Mioskowski E, Cherel Y and Farni J (1992). Stress in birds due to routine handling and a technique to avoid it. Am. J. Physiol. Regul. Integr. Comp. Physiol. 263: 775-781.
- Maret W and Sandstead HH (2006). Zinc requirements and the risks and benefits of zinc supplementation. J. Trace Elem. Med. Biol. 20: 3-18.
- Martin GA, Morris TB, Gehle MH and Harwood DG (1973). Force-molting by limiting calcium intake. Poult. Sci. 52: 2058.
- Maxwell MH (1993). Avian blood leukocyte responses to stress. World's Poult. Sci. J. 49: 34-43.
- Maxwell MH, Hocking PM and Robertson GW (1992). Differential leukocyte responses to various degrees of food restriction in broilers, turkeys and ducks. Br. Poult. Sci. 33: 177-187.
- McCormick CC (1984). Induction and accumulation of metallothionein in liver and pancreas of chicks given oral zinc: A tissue comparison. J. Nutr. 114: 191-203.
- McCormick CC and Cunningham DL (1984a). Forced resting by high dietary zinc: Tissue zinc accumulation and reproductive organ weight changes. Poult. Sci. 63: 1207-1212.
- McCormick CC and Cunningham DL (1984b). High dietary zinc and fasting as methods of forced resting. A performance comparison. Poult. Sci. 63: 1201-1206.
- McCormick CC and Cunningham DL (1987). Performance and physiological profiles of high dietary zinc and fasting as methods of inducing a forced rest: a direct comparison. Poult. Sci. 66: 1007-1013.

- McCowan B, Schrader J, DiLorenzo AM, Cordona C and Klingborg D (2006). Effects of Induced molting on the well-being of egg-laying hens. J. App. Anim. Welfare Sci. 9(1): 9-23.
- McDowell LR (1992). Minerals in Animal and Human Nutrition. Academic Press Inc. Harcourt Brace Jovanovich Publishers, San Diego, CA. New York
- McManus JFA and Mowry RW (1960). Staining methods. Histologic and Histochemical. New York: Hoeber. USA. pp. 423.
- McReynolds J, Kubena L, Byrd J, Anderson R, Ricke S and Nisbet D (2005). Evaluation of *Salmonella enteritidis* in molting hens after administration of an experimental chlorate product (for nine days) in the drinking water and feeding an alfalfa molt diet. Poult. Sci. 84: 1186-1190.
- McReynolds JL, Moore RW, Kubena LF, Byrd JA, Woodward CL, Nisbet DJ and Ricke SC (2006). Effect of various combinations of alfalfa and standard layer diet on susceptibility of laying hens to Salmonella enteritidis during forced molt. Poult. Sci. 85: 1123-1128.
- Medvedev KL, Moore RW, Woodward CL, Landers DA, Howard ZR, Byrd JA, Kubena LF, Nisbet DJ and Ricke SC (2002). Effects of alfalfa and feed deprivation molting methods on leucocyte percentage in laying hens. Poult. Sci. 81: 100 (Abstr).
- Mench JA (1991). Research note: Feed restriction in broiler breeders causes a persistent elevation in corticosterone secretion that is modulated by dietary tryptophan. Poult. Sci. 70: 2547-2550.
- Miller WJ, Clifton CM, Fowler PR and Perkins HF (1965). Influence of high levels of dietary zinc on zinc in milk, performance and biochemistry of lactating cows. J. Dairy Sci. 48(4): 450-453.
- Minear LR (1999). Southern States research, Richmond, VA. Personal communication.
- Mitchell ME (1970). Treatment of hypophysectomised hens with partially purified avian FSH. J. Reprod. Fertil. 2: 223-241.
- Mohamed AT (1987). Zinc oxide as a resting agent and its influence on layer performance. M. Sc. Thesis, Fac. of Agric. Cairo Univ. Egypt.

- Mohamed FR (1990). Studies on molting and its relation with egg production in the fowl. Ph. D Thesis, Fac. of Agric. Cairo Univ. Egypt.
- Moore RW, Park SY, Kubena LF, Byrd JA, McReynolds JL, Burnham MR, Hume ME, Birkhold SG, Nisbet DJ and Ricke SC (2004). Comparison of zinc acetate and propionate addition on gastrointestinal tract fermentation and susceptibility of laying hens to Salmonella enteritidis during forced molt. Poult. Sci. 83: 1276-1286.
- Moudgal RP and Razdan MN 1(984). Induction of ovulation in vitro in the hen: Dependency of the response to LH on age and rate of lay. J. Endocrinol. 106: 67-69.
- Mrosovsky N and. Sherry DF (1980). Animal anorexias. Science 207: 837-842.
- Munck A, Guyre P and Holbrook N (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrinol. Rev. 5: 25-44.
- Naber EC, Latshaw JD and Marsh GA (1984). Effectiveness of low sodium diets for recycling of egg production type hens. Poult. Sci. 63: 2419-2429.
- Nishiyama S, Futagoishi-Suginohara Y, Matsukura M, Nakamura T, Higashi A, Shinohara M and Matsuda I (1994). Zinc supplementation alters thyroid hormone metabolism in disabled patients with zinc deficiency. J. Am. Coll. Nutr. 13: 62-67.
- North MO and Bell DD (1999). Commercial Chicken Production Manual. 4th ed. Chapman Hall, New York. USA. pp. 861-878.
- Nys Y, Mayel-Afshar S, Bouillon R, Van-Baelen H and Lawson DEM (1989). Increases in calbindin D28k mRNA in the uterus of the domestic fowl induced by sexual maturity and shell formation. Gen. Comp. Endocrinol. 76: 322-329.
- Nys Y, Parkers CO and Thomasset M (1986). Effects of suppression and resumption of shell formation and parathyroid hormone on uterine calcium binding protein, carbonic anhydrase activity, and intestinal calcium absorption in hens. Gen. Comp. Endocrinol. 64: 293-299.

- Ocak N, Sarica M, Erener G and Garpoglu AV (2004). The effect of body weight prior to molting in brown laying hens on egg yield and quality during second production cycle. Int. J. Poult. Sci. 3: 768-772.
- Oguike MA (2004). Organ changes and laying performance of forced-molt layers. Ph.D. Dissertation. College of Animal Science and Animal Health Michael Okpara University of Agriculture Umudike. Abia State Nigeria.
- Oguike MA, Igboeli G, Ibe SN and Ironkwe MO (2005a). Physiological and endocrinological mechanisms associated with ovulatory cycle and induced-molting in the domestic chicken – A review. World's Poult. Sci. J. 61: 625-632.
- Oguike MA, Igboeli G, Ibe SN and Uzoukwu M (2004). Effect of day length and feed/water regime on induction of feather molt and subsequent laying performance in the domestic fowl. Int. J. Poult. Sci. 3: 507-512.
- Oguike MA, Igboeli G, Ibe SN, Ironkwe MO, Akomas SC and Uzoukwu M (2005b). Plasma progesterone profile and ovarian activity of forced-molt layers. Afr. J. Biotechnol. 4: 1005-1009.
- Ott EA, Smith WH, Harrington RB and Beeson WM (1966). Zinc toxicity in ruminants. I. Effects of high levels of dietary zinc on gains, feed consumption and feed efficiecy of lambs. J. Anim. Sci. 25: 414-418.
- Palafox AL and Ho-AE (1980). Effect of zinc toxicity in laying white leghorn pullets and hens. Poult. Sci. 59: 2024-2028.
- Palmer SS and Bahr JM (1992). Follicle stimulating hormone increases serum oestradiol-17 concentrations, number of growing follicles and yolk deposition in aging hens (*Gallus domesticus*) with decreased egg production. Br. Poult. Sci. J. 33: 403-414.
- Park SY, Birkhold SG, Kubena LF, Nisbet DJ and Ricke SC (2004a). Effects of high Zinc diets using Zinc propionate on molt induction, organs and postmolt egg production and quality in laying hens. Poult. sci. 83: 24-33.
- Park SY, Birkhold SG, Kubena LF, Nisbet DJ, Ricke SC (2004d). Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biol. Trace Elem. Res. 101: 147-63.

- Park SY, Kim WK, Birkhold SG, Kubena LF, Nisbet DJ and Ricke SC (2004c). Induced molting issues and alternative dietary strategies for egg industries in the United States. World's Poult. Sci. J. 60: 196-209.
- Park SY, Kim WK, Birkhold SG, Kubena LF, Nisbet DJ and Ricke SC (2004b). Using a feed – grade zinc propionate to achieve molt induction in laying hens and retain postmolt egg production and quality. Biol. Trace Elem. Res. 101: 165-179.
- Park SY, Woodward CL, Birkhold SG, Kubena LF, Nisbet DJ and Ricke SC (2004e). Influence of oxidation-reduction media reductants on Salmonella typhimurium growth kinetics response in an anaerobic atmosphere after initial pH adjustment and zinc compound addition. J. Rapid Meths. Auto. Microbiol. 12: 185-197.
- Pearce J, Jackson N and Stevenson MH (1983). The effects of dietary intake and of dietary concentration of copper sulphate on the laying domestic fowl: effects on some aspects of lipid, carbohydrates and amino acid metabolism. Br. Poult. Sci. 24: 337-348.
- Peczely P (1992). Hormonal regulation of feather development and molt on the level of feather follicles. Ornis Scandinavica 23: 346-354.
- Piersma T and Baker AJ (2000). Life history characteristics and the conservation of migratory shore birds. In: Behaviour and Conservation (ed. L.M. Gosling and W.J. Sutherland), Cambridge: Cambridge University Press. UK. pp. 105-124.
- Powell SR, Hall D, Aiuto L, Wapnir RA, Teichberg S and Tortolani AJ (1994). Zinc improves postischemic recovery of isolated rat hearts through inhibition of oxidative stress. Am. J. Physiol. 266: 2497-2507.
- Prasad AS (1984). Discovery and importance of zinc in human nutrition. Fed. Proc. 43: 2829-2834.
- Prasad AS (1993). Acquired zinc deficiency and immune dysfunction in sickle cell anemia. In nutrient modulation of the immune response. S. Cunningham-Rundles, ed., Marcel Dekker, New York, USA. pp. 393-410.
- Prasad AS (2004). Zinc deficiency: its characterization and treatment. Met. Ions. Biol. Syst. 41: 103-137.

- Prasad AS and Kucuk O (2002). Zinc in cancer prevention. Cancer Metastasis Rev. 21: 291-295.
- Prasad AS, Fitzgerald JT, Hess JW, Kaplan F, Pelen J and Dardenne M (1993). Zinc deficiency in elderly patients. Nutrition. 9: 218-224.
- Puschner B, Judy St. L and Francis DG. (1999). Normal and toxic zinc concentrations in serum/plasma and liver of psittacines with respect to genus differences. J. Vet. Diagn. Invest. 11: 522-527.
- Qin X and Klandorf H (1995). Effect of estrogen on egg production, shell quality and calcium metabolism in molted hens. Comp. Biochem. Physiol. 110: 55-59.
- Ramos RB, Fuentes MFF, Espíndola GB, Lima FAM and Freitas ER (1999). Efeito de métodos de muda forçada sobre o desempenho de poedeiras comerciais. Revista Brasileira de Zootecnia 28(6): 1340-1346.
- Reddy V, Malathi VK, Venkatarami and Reddy BS (2008). Effect of induced molting in male and female line broiler breeder hens by zinc oxide and feed withdrawal methods on post molt performance parameters. Int. J. Poult. Sci. 7 (6): 586-593.
- Ricke SC (2003). The gastrointestinal tract ecology of Salmonella Enteritidis colonization in molting hens. Poult. Sci. 82: 1003-1007.
- Ricke SC, Park SY, Moore RW, Kwon YM, Woodward CL, Byrd JA, Nisbet DJ and Kubena LF (2004). Feeding low calcium and zinc molt diets sustains gastrointestinal fermentation and limits Salmonella enterica serovar enteritidis colonization in laying hens. J. Food Safety 24: 291-308.
- Roberson RH and Francis DW (1979). The effect two molting methods on performance of Hyline and Shaver hens. Poult. Sci. 58: 1098.
- Roland Sr DA and Brake J (1982). Influence of pre-molt production on post-molt performance with explanation for improvement in egg production due to force molting. Poult. Sci. 61: 2473-2481.
- Roland Sr DA, Holcombe DJ and Harms RH (1977). Further studies with hens producing a high incidence of non-calcified and partially calcified egg shells. Poult. Sci. 56: 1232-1236.

- Rolon A, Buhr RJ and Cunningham DL (1993). Twenty-four-hour feed withdrawal and limited feeding as alternative methods for induction of molt in laying hens. Poult. Sci. 72: 776-785.
- Ross E and Herrick RB (1981). Forced rest induced by molt or low-salt diet and subsequent hen performance. Poult. Sci. 60: 63-67.
- Ruszler P and Novak CL (2006). Feeding hens during alternating a.m. and p.m.time blocks to induce zero egg production during the molt. J. Appl. Poult. Res. 15: 525-530.
- Ruszler PL (1996). The keys to successful force molting. Publication (revised). Virginia cooperative extension service. Blacksburg, VA. 408-426.
- Ruszler PL (1998). Health and husbandry considerations of induced molting. Poult. Sci. 77: 1789-1793.
- Ruszler PL and Minear LR (1997). Comparison of induced molts using periods of four vs ten days feed withdrawal. Poult. Sci. 76: 104 (Abstr).
- Sahin K, Sahin N, Kucuk O, Hayirli A and Prasad AS (2009a). Role of dietary zinc in heat-stressed poultry: A review. Poult. Sci. 88: 2176-2183.
- Sahin K, Smith MO, Onderci M, Sahin N, Gursu MF and Kucuk O (2005). Supplementation of zinc from organic or inorganic source improves performance and antioxidant status of heat-distressed quail. Poult. Sci. 84: 882-887.
- Sahin N, Tuzcu M, Ozercan I, Sahin K, Prasad AS and Kucuk O (2009b). Zinc picolinate in the prevention of leiomyoma in Japanese quail. J. Med. Food. 1368-1374.
- Said MW, Sullivan TW, Bird HR and Sunde ML (1984). A comparison of the effect of two force molting methods on performance of two commercial strains of laying hens. Poult. Sci. 63: 2399-2403.
- Salem, Amina A, Eman M, Abo-Etta and Nofal ME (2005). Productive and physiological performance of local laying hens as affected by force molting methods. Egypt. J. Appl. Sci. 20: 12-31.
- Salgueiro MJ, Zubillaga M, Lysionek A, Sarabia MI, Caro R, De Paoli T, Hager A, Weill R and Boccio J (2000). Zinc as essential micronutrient: A review. Nutr. Res. 20: 737-755.

- Samman B and Roberts DCK (1988). The effect of zinc supplements on lipoproteins and copper status. Atherosclerosis 70: 247-252.
- Sandhu MA, Rahman ZU and Rahman SU (2006). Dynamics of macrophages during second and third production cycles after zinc induced molting. J. Poult. Sci. 43: 286-295.
- Sandhu MA, Rahman ZU, Hasan IJ, Rahman SU and Yousaf MS (2008). Changes in luteinizing hormone-containing gonadotrophs after molting induced by fasting and zinc in laying hens (Gallus domesticus). J. Anim. Physiol. Anim. Nutr. 92: 668-676.
- Sandhu Ma, Rahman Zu, Riaz A, Rahman Su, Javed I and Ullah N. (2010). Somatotrophs and lactotrophs: an immunohistochemical study of *Gallus domesticus* pituitary gland at different stages of induced molt. Eur. J. Histochem. 54:123-127.
- Sandoval M, Henry PR, Ammerman CB, Miles RD and Littell RC (1997). Relative bioavailability of supplemental inorganic zinc sources for chicks. J. Anim. Sci. 75: 3195-3205.
- Scheideler S, Puthpongsiriporn U and Beck M (2002). Comparison of traditional fasting molt versus non-feed restriction low sodium molt diets and pre-molt photoperiod effects on molt and second cycle production parameters. Poult. Sci. 81: 22-23.
- Scheideler SE, Beck MM and LaBrash L (2003). Nonfeed withdrawal programs for laying hen molt. In Proc. Midwest poultry federation convention. 50-53.
- Scott JT and CR Creger (1997). The use of zinc as an effective molting agent in laying hens. Poult. Sci. 76: (Abstr).
- Scott JT and Creger CR (1976). The use of dietary zinc as an effective molting agent in laying hens. Poult. Sci. 55: 2089.
- Sekimoto K, Imai K, Suzuki M, Takikawa H, Hoshino N and Totsuka K (1987). Thyroxin-induced molting and gonadal function in laying hens. Poult. Sci. 66: 752-756.
- Selling W, Ahnefeld FW, Dick W and Fodor L (1975). The biological significance of zinc. Anaesthesist 24: 329-342.

- Senior BE and Furr BJA (1975). A preliminary assessment of the source of estrogen within the ovary of the domestic fowl (Gallus domesticus). J. Reprod. Fertil. 43: 241-247.
- Seo KH, Holt PS and Gast RK (2001). Comparison of *Salmonella enteritidis* infection in hens molted via long-term feed withdrawal versus full-fed wheat middling. J. Food Prot. 64: 1917-1921.
- Shankar AH and Prasad AS (1998). Zinc and immune function: The biological basis of altered resistance to infection. Am. J. Clin. Nutr. 68: 447-463.
- Sherry DF, Mrosovsky N and Hogan JA (1980). Weight loss and anorexia during incubation in birds. J. Comp. Physiol. Psychol. 94: 89-98.
- Shippee RL, Stake PE, Koehn U, Lambert JL and Simmons RW (1979). High dietary zinc or magnesium as forced-resting agents for laying hens. Poult. Sci. 58: 949-954.
- Siegel HS (1995). Stress, strains and resistance. Br. Poult. Sci. 36: 3-22.
- Silversides FG, Williams J and Merat P (1993). Effect of sex linked imperfect albinism in the chicken (sal-c) on plasma luteinizing hormone concentrations and early egg production. Br. Poult. Sci. J. 34: 1011-1019.
- Simkiss K (1961). Calcium metabolism and avian reproduction. Biol. Rev. 36: 321-367.
- Sonez MC and Von Lawzewitsch I (1997). Ultrastructural identification of pituitary cells in Nothura maculosa (*Tinamidae. Temminck*). Biocell. 21: 103-144.
- Sonez MC, Stancato MR and Von Lawzewitsch I (1995). Immunocytochemical identification of prolactin and somatotropin-containing cells in adenohypophysis of Nothura maculosa (*Tinamidae. Temminck*) during recess and active reproduction periods using human hormone antisera. Com. Biol. 13: 85-100.
- Southern LL and DH Baker (1983). Zinc toxicity, zinc deficiency and zinc-copper interrelationship in Eimeria acervulina-infected chicks. J. Nutr. 113: 688-696.

- Spears JW (1989). Zinc methionine for ruminants: Relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. J. Anim. Sci. 67: 835-843.
- Stevenson MH and Jackson N (1984). Comparison of dietary hydrated copper sulphate, dietary zinc oxide, and a direct method for inducing a molt in laying hens. Br. Poult. Sci. 25: 505-517.
- Sturkie PD (1976). Endocrine influences on lipid metabolism, in Avian Physiology, 3rd (Ed.), PD. Sturkie ed. Springer-Verlag, New York. USA.
- Su H, Silversides FG and Villeneuve P (1995). Ovarian morphology and follicular development in sex-linked imperfect albino (sal-s) and non albino hens before or after a induced-molt. Br. Poult. Sci. J. 35: 645-653.
- Sundaresan NR, Anish D, Sastry KV, Saxena VK, Mohan J and Ahmed KA (2007). Cytokines in reproductive remodeling of molting White Leghorn hens. J. Reprod. Immunol. 73: 39-50.
- Sundaresan NR, Anish D, Sastry KVH, Saxena K, Nagarajan K, Subramani J, Leo MDM, Shit N, Mohan J, Saxena M and Ahmed KA (2008a). High doses of dietary zinc induce cytokines, chemokines, and apoptosis in reproductive tissues during regression. Cell Tissue Res. 332: 543-554.
- Sundaresan NR, Saxena VK, Sastry KVH, Nagarajan K, Jain P, Singh R, Anish D, Ravindra PV, Saxena M and Ahmed KA (2008b). Cytokines and chemokines in postovulatory follicle regression of domestic chicken (*Gallus gallus domesticus*). Dev. Comp. Immunol. 32: 253–264.
- Sunder S, Panda G, Gopinath AK, Rama Rao NCS, Raju SV, Reddy MVLN, Rand M and Vijay Kumar Ch (2008). Effects of higher levels of zinc supplementation on performance, mineral availability and immune competence in broiler chickens. J. Appl. Poult. Res. 17: 79-86.
- Swanson MH and Bell DD (1975a). Force molting of chickens-IV Egg quality. Leaflet 2810. University of California, Davis, CA. USA.
- Swanson MH and DD Bell (1975b). Force molting of chickens-V Replacement programs. Leaflet 2811. University of California, Davis, CA. USA.

- Tanabe Y, Ogowa T and Nakamura T (1981). The effect of short-term starvation on pituitary and plasma LH, plasma estradiol and progesterone, and pituitary response to LH-RH in the laying hen (*Gallus domesticus*). Gen. Comp. Endocrinol. 43: 392-398.
- Tanaka K, Kamiyoshi M and Sakaida M (1974). Effects of progesterone on the hypothalamic gonadotropin releasing activity and on the pituitary gonadotropic activity in hens and cocks. Poult. Sci. 53: 1772-1776.
- Taylor TG (1965). Calcium-endocrine relationships in the laying hen. Proc Nutr. Soc. 24: 49-44.
- Thirunavukkarasu P, Moorthy M and Viswanathan K (2006). Body weight and egg production performance of induced molted White Leghorn layers. Int. J. Poult. Sci. 5: 996-1000.
- Thirunavukkarasu P, Moorthy M and Viswanathan K (2007). Body weight changes of single comb white leghorn layers at different ages during induced molt. Int. J. Poult. Sci. 6(12): 858-859.
- Thirunavukkarasu P, Moorthy M, Viswanathan K and Edwin SC (2009). Egg production performance of induced molted white leghorn layers. Tamilnadu J. Vet. & Ani. Sci. 5(3): 117-119.
- United Egg Producers (UEP) (2005). United egg producers animal husbandry guidelines for us. laying flocks. 2nd (Ed). UEP, Alpharetta, GA.
- Underwood EJ (1977). Zinc. In Trace elements in human and animal nutrition. 4th ed. Academic Press, New York, NY. USA. pp. 196-242.
- Vallee BL and Auld DS (1980). The metallobiochemistry of zinc enzymes. A. Meister, (Ed). Adv. Enzymol. pp. 283-429.
- Van Tienhoven A (1981). Neuroendocrinology of avian reproduction with special emphasis on the reproductive cycle of the fowl (*Gallus domesticus*). World's Poult. Sci. J. 37: 155-176.
- Vanmontfort D, Berghman LR, Rombatus L, Verhoeven G and Decuypere E (1994). Changes of immunoreactive inhibin, follicle-stimulating hormone, luteinizing hormone and progesterone in plasma after short-term food

deprivation and during ovulatory cycle of the domestic hen. Gen. Comp. Endocrinol. 95: 117-124.

- Venkata R, Malathi VK and Venkatarami BS (2008). Effect of Induced Molting in Male and Female Line Broiler Breeder Hens by Zinc Oxide and Feed Withdrawal Methods on Post Molt Performance Parameters. Int. J. Poult. Sci. 7(6): 586-593.
- Verheyen G (1986). Endocrinological backgrounds of different methods for induced egg laying stop and molt in hens. Ph.D. thesis, Catholic University of Leuven (Belgium).
- Verheyen G, Decuypere E, Chiasson RB, Vervloesem J, Kuhn ER and Michels H (1987). Effect of exogenous luteinizing hormone on plasma concentration of progesterone and oestradiol in relation to the cessation of egg laying induced by different molting methods. J. Reprod. Fertil. 81: 13-21.
- Verheyen G, Decuypere E, Kuhn ER, Fontaine G. and De Groóte, G (1983). Cessation of egg production in chickens by induction. Effect of different methods on some production parameters and on thyroid hormone concentrations, prolactin, Ca, P, Na and protein in the blood serum. Rev. de Agric. 36: 1535-1559
- Verheyen G, Helsen J and Decuypere E (1990). Accumulation of zinc in egg yolk, ovarian follicles and organs after forced resting by high dietary zinc. Br. Poult. Sci. 31(1): 147-154.
- Virden WS, Yeatman JB, Barber SJ, Zumwalt, CD, Ward TL, Johnson AB and Kidd MT (2003). Hen mineral nutrition impacts progeny livability. J. Appl. Poult. Res. 12: 411-416.
- Waddington D, Perry MM, Gilbert AB and Hardie MA (1985). Follicular growth and atresia in the ovaries of hens (Gallus domesticus) with diminished egg production rates. J. Reprod. Fertil. 74: 399-405.
- Walker RF and Frawley LS (1977). Gonadal function in underfed rats: II. Effect of estrogen on plasma gonoadotropins after pinealectomy or constant light exposure. Biol. Reprod. 17: 630-634.
- Wang LC and Busbey S (2005). Images in clinical medicine. Acquired acrodermatitis enteropathica. N. Engl. J. Med. 352: 1121.

- Webster AB (2003). Physiology and behavior of the hen during induced molt. Poult. Sci. 82: 992-1002.
- Wedekind KJ and Baker DH (1990). Zinc bioavailability in feed-grade sources of zinc. J. Anim. Sci. 68: 684-689.
- Wedekind KJ, Hortin AE and Baker DH (1992). Methodology for assessing zinc bioavailability: Efficacy estimated for zinc-methionine, zinc sulfate, and zinc oxide. J. Anim. Sci. 70: 178-187.
- Wells JW, Walker MA, Culbert J and Gilbert AB (1985). Comparison of the response in vivo to luteinizing hormone of the granulosa of six follicles from the ovarian hierarchy in the chicken (Gallus domesticus). Gen. comp. Endocrinol. 59: 369-374.
- Whitehead C and Shannon D (1974). The control of egg production using a low-sodium diet. Br. Poult. Sci. 15: 429-434.
- Wight PAL, Dewar WA and Saunderson CL (1986). Zinc toxicity in the fowl: ultrastructural pathology and relationship to selenium, lead and copper. Avian Pathol. 15: 23-28.
- Williams JB and Sharp PJ (1977). A comparison of plasma progesterone and luteinizing hormone in growing hens from eight weeks of age to sexual maturity. J. Endocrinol. 75: 447-448.
- Williams JB and Sharp PJ (1978). Ovarian morphology and rates of ovarian follicular development in laying broiler breeder and commercial egg producing hens. Br. Poult. Sci. J. 19: 387-395.
- Williams JB, Etches RJ and Rzasa J (1985). Induction of a pause in laying by corticosterone infusion or by dietary alternations: effects on the reproductive system, food consumption, and body weight. Br. Poult. Sci. 26: 25-34.
- Wilson HR, Fry JL, Harms RH and Arrington LR (1967). Performance of hens molted by various methods. Poult. Sci. 46: 1406-1412.
- Wilson SC and Cunningham FJ (1980). Modification by metyrapone of the open period for pre-ovulatory LH release in the hen. Br. Poult. Sci. 21: 351-361.
- Wilson SC and Sharp PJ (1973). Variation in plasma LH levels during ovulatory cycle of the hen (*Gallus domesticus*). J. Reprod. Fertil. 35: 561-564.

- Wingfield JC, Maney DL, Breunerj CW, Jacobss D, Lynn M, Ramenofskya N and Richardson DRD (1998). Ecological bases of hormone behavior interactions: the emergency life history stage. Am. Zool. 38: 191-206.
- Wintergerst ES, Maggini S and Hornig DH (2007). Contribution of selected vitamins and trace elements to immune function. Ann. Nutr. Metab. 51:.301-323.
- Wolford JH (1984). Induced molting in laying fowls. World's Poult. Sci. J. 40: 66-73.
- Wolford JH and RK Ringer (1962). Adrenal weight, adrenal ascorbic acid, adrenal cholesterol and differential leucocyte counts as physiological indicators of "stressor" agents in laying hens. Poult. Sci. 41: 1521-1529.
- Woodward CL, Kwon YM, Kubena LF, Byrd JA, Moore RW, Nisbet DJ and Ricke SC (2005). Reduction of *Salmonella enterica* serovar Enteritidis colonization and invasion by an alfalfa diet during molt in Leghorn hens. Poult. Sci. 84: 185-193.
- Yoshimura Y and Tamura T (1985). Histological and histochemical observations on the atretic follicles induced by adenohypophysectomy in the hen. J. Poult. Sci. 22: 134-141.
- Yoshimura Y, Okamoto T and Tamura T (1989). Enzyme histochemical observations on the ovarian small follicles in relation to the mechanism of atresia in laying hens (*Gallus domesticus*). Jpn. Poult. Sci. 26: 10-16.
- Yousaf M (1998). Comparative study of induced molt methods in relation to plumage renewal and productive performance of layers under cage and litter floor systems. Ph.D. thesis, Department of Poultry Science, University of Agriculture, Faisalabad, Pakistan.
- Yousaf M (2004). Influence of different copper and aluminium levels on feather renewal and production characteristics of the layers in the second production cycle. Research project report submitted to Pakistan Science Foundation, Islamabad, Pakistan.
- Yousaf M (2006a). Influence of different copper and aluminum levels on organ weights, feather renewal and production performance of molted layers. Pak. J. Arid Agri. 9: 35-39.

- Yousaf M (2006b). Induced molting: Tips for success. Poult. Int. 45(4): 36-40.
- Yousaf M and Ahmad N (2006). Effects of housing systems on productive performance of commercial layers following induced molting by Aluminium oxide supplementation. Pak. Vet. J. 26: 101-104.
- Yousaf M and Chaudhry AS (2008). History, changing scenarios and future strategies to induce molting in laying hens. World's Poult. Sci. J. 64: 65-75.
- Yousaf M, Ahmad N, Akram M and Zia-Ur-Rehaman (1998). Changes in the concentration of various blood plasma parameters and hormones as influenced by induced molt in White Leghorn layers. Pak. Vet. J. 18: 36-42.
- Yousaf M, Ahmad N, Sarzamin K and Ahmad T (2008). Effect of non-feed withdrawal induced molting techniques on feather molt scoring and egg production of caged hens. Sarhad J. Agric. 24: 31-37.
- Zeelen HHM (1975). Technical and economic results from forced molting of laying hens. World's Poult. Sci. J. 31: 57-67.
- Zimmermann NG, Andrews DK and McGinnis J (1987). Comparison of several induced molting methods on subsequent performance of single comb white Leghorn hens. Poult. Sci. 66: 408-417.