EFFECT OF FURAZOLIDONE-TREATMENT ON REPRODUCTIVE FUNCTIONS, SERUM ESTRADIOL AND GONADOTRAPHIN IN FEMALE *RHODE ISLAND RED* LAYER BIRDS

BY

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DEDICATED

TO MY

MOTHER

FOR HER

Prays, Love, Patience and Sacrifices for me.

CERTIFICATE AND APPROVAL

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

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ABSTRACT

The current study was carried out to determine the effects of furazolidone-administration on reproductive performance of female Rhode Island Red birds. Birds were allocated to four groups and each group consisting of eight female and one male. Furazolidone was administered orally at the prelaying age at doses of control 0mg/kg feed, group I 200mg/kg feed, group II 400mg/kg feed and group III 800mg/kg feed for period of 5 weeks (15-20weeks of age) and birds kept for another four weeks on furazolidone free diet. At the age of 24 weeks birds were sacrificed.

Furazolidone produced dose dependent effects on body weight of the birds. However, in 2^{nd} week of furazolidone-administration the body weight of group III was significantly ($P<0.05$) reduced. In the 3^{rd} and 4^{th} week, furazolidone-treatment resulted in significant ($P<0.05$; $P<0.02$) decrease in body weight of group II and III respectively. Similar, highly significant ($P<0.01$) trend in reduction in body weight of the group II and III was observed during 5^{th} week of treatment. There was no difference in the body weight of group I compared to control birds at any stage of furazolidone-administration. This reduction in body weight was also observed during 1^{st} and 2^{nd} week of furazolidone cessation that significantly ($P<0.01$;$P<0.02$) lower in group II and III compared to control. During 3^{rd} week of the mean body of group II became similar to control whereas in group III it remained significantly ($P<0.02$) lower compared to control. Body weights of all groups were comparable in 4^{th} week.

Feed conversion ratio (FCR) was comparable in group I and II to control birds during Ist, 2^{nd} and 3^{rd} week of furazolidone treatment. FCR in group III was comparable to control birds at the end of first week but reduced significantly ($P<0.05$) during 2^{nd} and 3^{rd} week of furazolidone-treatment. There was significant ($P<0.02$) decrease in FCR of group II and III during 4^{th} and 5^{th} week of furazolidone-treatment

Furazolidone-treatment decreased ($P<0.0001$) serum cholesterol level in all treated birds. Serum cholesterol reduced significantly ($P<0.02$; $P<0.01$) during Ist and 2^{nd} week of furazolidone-treatment in group II and III. Serum cholesterol concentration was adversely affected ($P<0.001$)during 3^{rd}-5^{th} week of experiment in group II and III. After withdrawal of furazolidone serum cholesterol level remained significantly ($P<0.05$; $P<0.02$; $P<0.01$; $P<0.001$) lower in all the treated groups compared to control. In 4^{th} week cholesterol level was comparable in all groups except group III.

Serum estradiol concentration reduced ($P<0.0001$) in all groups by the furazolidone-administration. Serum cholesterol level was similar in all groups at the end Ist week but significant ($P<0.05$; $P<0.02$; $P<0.001$) reduction was observed in group II and III during 3^{rd} and 5^{th} week. Serum estradiol concentration remained significantly ($P<0.01$; $P<0.001$) lower in group II and III at 2^{nd} week of furazolidone cessation and approached to normal at 4^{th} week.

Furazolidone-treatment decreased ($P<0.0368$) serum LH level in all groups. Lower dose of furazolidone caused no change in serum LH levels. However, a significant reduction was observed in serum LH level with higher concentration during 3^{rd} and 5^{th} week of furazolidone-administration. Even after fourth week of furazolidone withdrawal, serum LH remained significantly ($P<0.05$; $P<0.001$) lower in all the treated groups.

Furazolidone-administration resulted in delay in egg laying and reduced ($P<0.0032$) egg production in all treated groups. A significant increase ($P<0.0001$) in egg production was observed with increasing age of the birds. The weight and volume of

the eggs increased ($P<0.0001$; $P<0.001$) with the advancement of age. Furazolidone did not effect the size of the egg.

The total ovarian weight with large follicles was not affected by furazolidone-administration but ovarian weight with only small follicles ($P<0.01$) and ovarian volume with small follicles were reduced ($P<0.02$; $P<0.01$) in group II and III. Ovarian size was comparable to control birds.

The length of oviduct significantly decreased ($P<0.05$; $P<0.01$) in group II and III compared to control but did not effect the weight and volume of the oviduct.

Furazolidone-administration did not significantly ($P>0.05$) effect the secondary sex organs (Wattles and Comb) and liver weight of the birds.

Furazolidone also induced various changes in oocyte proliferation. Furazolidone-administration caused significant ($P<0.05$; $P<0.01$; $P<0.001$) decrease in mean number of oocyte diameter in the range of 101-400μm in all the treated birds. The mean number of oocyte diameter in the range of 401-600μm decreased ($P<0.02$; $P<0.01$) in group II and III only. The oocytes with diameter in the range of 601-700μm and 701-800μm were not found in treated birds.

The mean number of follicles with granulosa layer thickness 1-10μm decreased in group II and III. While, follicles with granulosa layer thickness in the range of 11-50μm did not differ from control in all groups. The mean number of oocytes with nuclei diameter in the range of 21-60μm decreased in treated bird. The mean number of oocyte with nuclei in the range of (60-80μm) were only found in control group

Furazolidone-treatment caused significant reduction ($P<0.05$; $P<0.01$) in mean number of yolky follicles of diameter in the range of 1-10mm in treated groups II and III. The mean number of yolky follicles diameter in the range of 21-25mm decreased in group II and III.

Furazolidone-administration also altered the organization of ovarian structures. The ovary of treated birds showed disrupted zona pellucida, loose stromal tissue, no cytoplasmic extension and small size lesser number of follicles on the cortical region.

CHAPTER

1

INTRODUCTION

INTRODUCTION

Livestock is an important sector of agriculture in Pakistan, which accounts nearly 37% of agriculture value added and 9% of the GDP. Livestock also contribute nine percent of the overall export earnings of the country. Over 30-35 million rural populations of the country are engaged in livestock raising. In 1999-2000 commercial layers population was 13.9 million and rural poultry layers was 71.2 million (Economic Survey, 1999-2000).

Infectious diseases are a constant threat to the poultry industry all over the world. Different antibacterial agents are routinely administered at prophylactic and therapeutic level to apparently healthy birds in order to prevent the possible losses from different latent or sub-clinical bacterial infections. Furazolidone is one of commonest and cheapest antibacterial agent administered in poultry feed. For poultry it is recommended in the feed at a concentration of 0.04 % (Brander *et al.*, 1991). However, due to narrow margin between safe and toxic levels, its prolonged administration may lead to toxicosis. Furazolidone chemically, is N-(5-nitro-2-furfurylidene)-amino–2-oxazolidonone (Ali, 1999) and occurs as an odorless yellow crystalline powder of bitter after tastes belonging to nitrofurans. The crystals darken under strong light and are decomposed by alkali. Both the antibacterial activity and the animal toxicity of nitrofurans depends on the presence of a nitro group (No2) located at the 5 position of the furan ring (Botsoglou *et al.*,1989; Brander *et al.*, 1991; Radotits *et al.*, 1994).

Furazolidone was approved in 1953 to widely use in animal including poultry for the treatment of certain bacterial and protozoal infections and in 1957 as a human systemic medicinal for the world wide use (Federal Register, 1976b; Bryan, 1978;

Murphy and Nelson, 1983; North, 1984; Zheng, 1984, 1985; Phillips and Hailey, 1986; Fraser *et al.*, 1991; Ali, 1999). In poultry furazolidone due to its antibacterial activity is used in feed for the treatment of certain diseases of bacterial origin notably fowl typhoid (Dutta *et al.*,1993), paratyphoid, pullorum, colibaccillosis, blackhead (histomoniasis), nonspecific enteritis (blue comb mud fever), Ulcerative enteritis (Quail disease), Synovitis, paracolon infection effectiveness of furazolidone in fowl cholera was also confirmed in young chicken and camphylobacter hepatitis (Federal Register, 1976a; North, 1984; Ashton, 1990; Bartlet *et al.*, 1990; Fraser *et al.*, 1991). It is also observed that resistance to furazolidone of different isolates of E. coli is comparatively quite less than to any other antibacterial agent (Tariq, 1989). Furazolidone is commonly used in man as an antidiarrohearic drug (Phillips and Hailey, 1986). In spite of its antifertility action furazolidone still used in man and animals (Nissim, 1957; Hagenas and Rtizen, 1978). It has some immunoprotective properties and patients with AIDS are known to be particularly susceptible to opportunistic and other infections, this drug may be effective in combating some of these infections. (Bychkova *et al.*, 1993; Dionisio *et al.*, 1995) Furazolidone was also found to be an effective therapeutic agent against gastrointestinal infections, brucellosis intestinal infections of undetermined etiology and peptic ulcer disease for more than 25 years in humans (Paul and Paul, 1964, 1966; Miura and Reckendorf, 1967; Bryan, 1978; Zheng and Wang, 1992) and as a prophylactic feed additive in livestock (Phillips and Hailey, 1986; Babu *et al.*,1994; Rossi *et al.*, 1995), prevention and treatment of coccidiosis infestation (North, 1984; Fraser *et al.*, 1991). As a feed additive, the compound has been approved for use in chickens to enhance growth and feed efficiency (Federal Register, 1976a). However high dietary levels, prolonged

pharmacology and therapeutic application of furazolidone in poultry produces several untoward and toxicological actions too which include, biventricular dilatation, bile duct hyperplasia, portal fibrosis, cardiomyopathy round heart disease (RHD), loss of weight gain, high mortality (Czarnecki *et al.*, 1974; Jensen *et al.*, 1975; Staley *et al.*, 1978; Ali and Bertlet, 1979, 1980, 1981, 1982a, 1982b, Czarnecki and Evanson, 1980; Good and Czarnecki, 1980; Ali, 1983; Van Vleet and Ferrans, 1983b Ali *et al.*,1984b; Reed *et al.*, 1987; Ali *et al.*, 1988; Czarnecki 1989; Bahgat *et al.*, 1990; Fraser *et al.*, 1991). In early chick embryo it was investigated that furazolidone injection directly into yolk sac at early stage of incubation, affects both growth and levels of glycogen in dosage dependent and time-dependent without causing any gross abnormalities (Czarnecki and Sujarit,1979). Cohen (1978) reported the symptoms of acute toxicity of furazolidone in humans, nausea, emesis, occasional diarrhea, abdominal pain, and bleeding. In frequently use, there have been reports of an idiosyncratic or hypersensitivity reaction such as pneumonitis (Collins and Thamas, 1973; Jirasek and Kalensky, 1975). It has been reported that most nitrofurans are mutagenic and carcinogenic by damaging the building unit of the cell namely the deoxyribonucleic acid of bacterial and animal cells (The New Straits Times Press Beuhed, 1996).

In male ducklings regression in cardiac lesions, decreased mortality, progressive decrease in ascites and increased in body weight was observed by Van Vleet and Ferrans (1983a). Arbid *et al.*(1990) observed that furazolidone treatment significantly altered growth, decreased feed consumption, pronounced atrophy of egg laying. and also caused a decreased in serum total protein. Webb and van Vleet (1990) studied the ultra structural alteration, cytoplasmic vacuolation of sertoli cells. They also founded

the decreased feed consumption and nervous signs including hyper excitability, incordination and seizures and gross pathological lesions were cordiomegaly, thinning of myocardium, pericardial effusion, pulmonary edema and congestion, ascites and testicular enlargement. Noorani *et al.* (2001) reported that furazolidone shown significantly lower feed consumption, body weight, testes weights and testes volume in the reversible fashion within four week after the withdrawal of the drug in Japanese quails. Wan and Yan (1983) reported that excessive mixing of furazolidone in feed for prevention of coccidiosis resulted in poisoning of flock. Orr *et al.* (1986) recorded that furazolidone toxicity exacerbated by other feed additives in the diet. In broiler chicks furazolidone treatment resulted in breathing difficulties and nervous signs (Salyi *et al.*, 1986). There was eosinpenia and slight lymphocytosis, prolonged nephritis and hepatitis at high concentration of furazolidone (Nemteanu *et al.*, 1979). Agate *et al.* (1983) concluded that furazolidone administration in white leghorn cockerels decreased serum globulin and body weight.

Furazolidone affects the metabolism of calcium and phosphorus in fowl and also reduced hemoglobin and iron in blood of white leghorn (Avramenko, 1983; 1985). Oyejide *et al.* (1983) administered furazolidone in white leghorn and observed decrease in body and liver weights of pullets and serum globulin of cockerels. It was investigated that an increase in serum aspartate aminotransferase (AST) alanine aminotransferase (ALT) and alkaline phosphates and a decline in serum total protein, decreased in liver weight and degenerative changes in hepatocytes were also observed (Arbid, 1990).

In female Japanese Quail furazolidone caused reduction in egg production in dose dependent manner and duration of treatment. In affected birds depressed production

continued until the diets containing furazolidone were removed Furazolidone also reduced the hatchability at high level of doses and time of duration of exposure to drug, ascribed to an increase in the number of infertile eggs. There was decreased in embryonation in these eggs but these changes were reversible and very slow (Dixon *et al.*, 1992). Ali *et al* (1986) noted dose dependent affects of furazolidone on egg production, LH and prolactin in female turkeys and furazolidone treatment also decreased the LH concentration in male turkeys, however prolactin concentration was unaffected by any dose of the drug used. LH and testosterone plasma concentrations significantly decreased in male turkeys (Ali *et al.*, 1988) and chickens (Andrabi *et al.*, 1998). The egg production was decreased by furazolidone treatment in turkey (Ali *et al.*,1987),but at same therapeutic dose level it did not alter the egg production in domestic fowl (Fracis and Schaffner,1956; Kondra and Guenter, 1968).

Ullah *et al* (1998) also studied that ovaries of Japanese quails treated with different doses of furazolidone decrease in the size, weight and become flattened with small follicles. Magnum, Isthmus and Uterus had decreased area, height and number of mucosal folds. Microscopically, furazolidone treated groups showed decrease height in mucosal gland cells and centrally located nuclei with foamy cytoplasm, these changes were reversible. Arbid *et al.* (1990) observed that furazolidone treatment for fourteen days also caused degenerative changes i.e ovaries become drastically atrophied after second week's treatment and in immature Japanese quail birds become misshapen. Histologically there were mass of immature follicles with a less vascularized medulla, reduced length and diameter.

Chickens subjected to additional artificial illumination significantly increased the cholesterol level in the plasma (Polonis, 1982) which is a precursor for steroid

hormones. During oviduct development, epithelial cell proliferation was preceded by sharp decrease in plasma progesterone and most of cell division completed during this phase. At the same time cessation of cell proliferation occurred with increase in estradiol. Progesterone might be one of the key signals that regulate the initiation of oviduct growth in quail (Pageaux *et al.,* 1984). It was investigated that high level of furazolidone altered growth, decreased feed consumption and resulted in higher mortality. Furazolidone also exhibits mutagenic activity (Mccalla and Voutsinos, 1974; Blijleven *et al.,* 1977; Tatsumi *et al.,* 1978) and possibly carcinogenic activities (Food and drug Administration, 1976). Luteinizing hormone concentration decreased progressively in a time and dose dependent fashion following treatment (Ali *et al.,* 1988) in male turkey. Sperm motility in vitro also affected by furazolidone (Deibel *et al.,* 1976) and in chickens furazolidone reduced the feed consumption, body weight, and feed efficiency, decrease the number of sperm in the vas-Deferens, seminiferous diameter and leyding cell nucleus diameter in male chickens (Andrabi *et al.,* 1998). In duckling furazolidone at doses of 250-750mg/kg in the feed caused ultrastructural cystic testicular degeneration. These included cytoplasmic vacuolations of Sertoli cells, cytolysis and desquamation of the rounded cells into the tubular lumen, and dilated seminiferous tubules (Webb and Van Vleet, 1991).

In domestic hen, several million follicles are present (Hutt, 1949) of which several thousand are macroscopically visible (Pearl and Schopes,1921). Follicles up to 6mm or so small yolky follicles are susceptible to atresia (Fell, 1923) follicles larger than about 8mm normally grow and ovulate. Maida *et al.* (1979) this was investigated that σ5-3B-hydroxysteroid dehydrogenase activity present in granulosa cells of follicles having size larger than 5mm and steroidogenesis occurs in granulosa cells for only the

last 7-8 days of their life in the pre-ovulatory follicle.. Differences in follicular populations in different regions of ovary was studied by Waddington *et al* (1988), that anterior portion of ovary contains more follicles of >8 mm in diameter and almost certain to ovulate. They also concluded that during laying period of hen ovulation must be from the anterior part of ovary. At peak laying periods the ovary of the domestic hen contained 30-100 small yolky follicles with diameter varies between 1 and 8mm in size. These follicles decreased in number with increasing in size of the follicles. The small yolky follicles between 5 and 25mm were atretic. The normal fate of small yolky follicles in the birds with a high rate of lay, indicting high incidence of atresia and reduction in number of follicles. Ovulation rate appear to be product of two complimentary mechanisms, on for the initiation of growth and the other controlling the rate of at which the small yolk follicles are through atresia.

Avian ovary produces steroid hormones like all mammal (Gilbert. 1971; Lofts and Murton, 1973; Murton and Westwood, 1977) steroid dehydrogenesis in the interstitial and granulosa cells of the chicken ovary was also reported (Baillie *et al.*, 1966; Gilbert, 1971; Loft and Murton, 1973; Murton and westwood, 1977). Estrogen was considered to be most probably produced by the thecal interstitial cells (Marshall and Coombs,1957; Lofts and Murton 1973; Huang and Nalbondov, 1978) but Chieffi and Botte (1965) argued that estrogens were produced by the granulosa cells. Lofts and Murton (1973) has indicated differences in ovarian steroid dehydrogenase activity between breeding and non-breeding conditions. Kohler *et al.* (1969) studied cytodifferentiation of the ovalalbumin glands in the chick oviduct by inducing diethylstilbestrol. There was marked perivascular edema and interstitial migration of mononuclear cells.

The estradiol, concentrations in the peripheral plasma of the hen show peaks at 18-22h and at 2-6h prior to ovulation (Peterson and Common, 1972). Senior and Cunningham (1974) reported an increase in estradiol concentrations 8h prior to ovulation with a peak concentration at 6h prior to the LH peak. Lague *et al.* (1975) determined the estrogen and progesterone precede ovulation by 4-7 hours and estrogen peaks not to be related to ovulation. Estrogen induced histological, ultrastructural, and biochemical chains during cytodifferentiation of the ovalalbumin-secreting gland in chick oviduct. Mitotic activity in the immature mucosal epithelium increases within 24hr and glands began to develop days 2-4 as bud like invagination into the sub-epithelial stroma (Kohler et al.,1969). Estrogen may play very important role in the growth and development of ovarian follicles (Sturkie,1965; Lofts and Murton, 1973) and also help in growth of oviduct (Strukie,1965).

In avian LH might have three peaks prior to ovulation (Imai and Nalbandov, 1971) but when LH concentrations were determined by Radioimmunoassay, there was only one peak of LH at about 4-7h prior to ovulation (Furr *et al.*, 1973). Cunningham and Furr (1972) found a small peak at about 20-23h prior to ovulation. They also state that the rise in progesterone either preceded or occurred simultaneously with the LH increase but that the rise in LH never proceeded the rise in progesterone. Three significant LH peak were detected in the plasma. Two occur 13 and 8h prior to ovulation and are essential for ovulation. The first of the three peaks occurs 21h prior to ovulation. It occurs immediately after the ovum passes from the oviduct into the uterus (Nelson *et al.*, 1965).

It was also reported that LH release occurs eight hour (Rothchild and Fraps, 1949) or 11-14 hour prior to ovulation (Van Tienhoven *et al.*, 1954). Ali *et al.*, (1988) reported

concentration of LH decreases progressively in a time and dose dependent manner following furazolidone treatment. They noted the highest dose of FZ (20mg/kg) reduces LH level by 91 % in male turkeys and also in female turkeys (Ali *et al.,* 1987). Plasma progesterone peak concentration generally coincides with that of the LH (Pageaux *et al.,* 1984). How FSH and LH regulate ovarian steroidogenesis in bird is not well understood. In *Gallus domesticus* there was a steady increase in the plasma LH concentration and estrogen but not of progesterone at the onset of ovarian development (Senior, 1974; Senior and Cunningham, 1974; Wilson and Sharp1975; Williams and Sharp 1977; Silver et al., 1978). The concentration of estrogen is maximum 2-3 weeks before Ovulation, which may due to rapid formation and growth of follicles (Peterson and Commen, 1972). Most of the work has been done to study the patterns of steroidogenesis in maturing the ovarian follicles in the domestic hens (Kumagai and Humma, 1974; Shahabi *et al.,* 1975; Huang *et al.,* 1979). It is observed that as the follicle enlarges, it produces increasing quantities of estrogen but after it becomes the 2nd largest follicle in hierarchy, aromatase activity declines, progesterone and testosterone synthesis increases (Etches and Cunningham, 1976). Estrogen is chiefly produced by the small yolky follicles (Etches, 1983). The plasma concentration of estradiol is higher in laying hens as compared to broody hens (Bedrak *et al.,* 1981) and turkey (Porter *et al.,*; 1989). Thecal and granulosa cells of the developing follicles present all the histochemical and ultrastructural details of steroid secreting cells (Boucek and Savard, 1970; Sayler et al., 1970 and Guraya, 1976) and are presumably the source of circulating estrogen and progesterone. Boucek and Savard (1970) reported that histochemical evidence for steroidogenic

cells in the stroma of the hen ovary do not support a separate interstitial tissue. These cells are actually thecal cells and prefers the term a thecal gland. (Dahl, 1971).

The present study is mainly concerned with the effect of chronic oral furazolidone-administration on the reproductive performance in layer hen (*Road Island Red)*. The work reported in livestock has been done mainly on male reproductive efficiency in birds and mammals (Ali et al., 1984b; Mustafa *et al.*, 1987). Scant work on the concentrations of steroids hormones and histomorphological changes in the gonads of female birds caused by furazolidone treatment has been reported. In view of this, experiment was carried out on layer hen given furazolidone-treatment to see its effects on body growth, feed efficacy, cholesterol concentration in serum, egg production and other morphometeric changes including LH and estrogens secretion were also studied.

CHAPTER

2

MATERIALS AND METHODS

MATERIALS AND METHODS

BIRDS AND EXPERIMENTAL DESIGN

A total of 36 birds (Rhode Island Red; a hybrid developed from Asiatic black-red fowls of Shanghai, Malay and Java types, bred on the farms of Rhode Island Province in America) at 13 week of age (prelaying age) were brought from Government poultry farm Kotli (Azad Kashmir). The birds were allowed to acclimatize their surroundings for two weeks. Then birds were divided randomly into four groups. Each group comprised of eight female and one male bird. The birds were kept at the Animal House of Quaid-I-Azam University, Islamabad, under the standard condition of management and feeding as advised in (Rose Parent stock management manual,1995).

At the age of 15 weeks the birds were randomly assigned to receive, 0mg, 200mg, 400mg and 800mg of furazolidone (Furazal, 24.4%furazolidone, Hillton Pharma pakistan) per kg feed daily. The groups were designated according to their diet.

Control group	fed a diet without furazolidone
Group I	fed a diet containing 200mg/kg feed per day
Group II	fed a diet containing 400mg/kg feed per day
Group III	fed a diet containing 800mg/kg feed per day

The standard poultry feed (Saddiq Brothers poultry meal) was fed to the experimental birds. Total duration of experiment was 9 weeks but at age 20 weeks oral administration of medicated feed was ceased and then experimental birds were rared on diet, which was free from furazolidone treatment. During this period the birds were subcutanously vaccinated twice against New Castle disease (ND) The body weight of

all groups was measured by weighing each birds individually on weekly basis. The amount of feed for birds adjusted to keep their body weight close to the target body weights. This was necessary because uniformity of sexual maturity is more likely to be disrupted in the period of 15-22weeks, if smooth transition of body weight gain and group body weight uniformity does not approximate closely to that of a standard body weight (Ross 308 parent stock management manual, 1995).

The feed used during the whole experiment was analyzed weekly by the feed testing laboratory, Poultry Research Institute (PRI) Rawalpindi. The analyzed feed contained 89.48 % dry matter, 10.53 % moisture, 14.66 % crude protein, 7.63 % crude fat, 4.38 % crude and 9.13 % total mineral (Ash) and 5-15 PPB aflatoxin. The analysis of these components of feed is so important as crude protein has a vital impact on fertility (Ross Parent stock management manual 1995). Sodium chloride and aflatoxin both have antifertility effects besides producing various lesions which resemble to that of furazolidone toxicity in different avian species (Siller *et al.*, 1972; Whitehead *et al.*, 1985; Webb and van Vleet, 1990, 1991; Arshad *et al.*, 1992).

Birds were frequently observed to detect any clinical alterations that might occur and those that died during the experiment were subjected to postmortem examination.

TEMPERATURE AND LIGHTING SCHEDULE

The birds were housed in groups on floor on deep litter system using dry rice hulls, within individual previously cleared and fumigated pens of 6X4 feet size. Each pen was separated by 3 feet high partition to avoid the mixing of birds. Experimental birds were kept at control temperature of 22-24C^0 and a relative humidity of 54-78% Bartlett *et al.*, 1990). Egg production by layer birds directly depends upon

photoperiod. Birds were provided daily period of 12 hours light upto 15 weeks of age, there after photoperiod was gradually increased so that it reached 17 hours daily at end of 18th week (SB layer management program). This photoperiod was maintained throughout 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/4cm^3 (Anjum, 1998).

BLOOD COLLECTION

Blood samples were collected weekly in sterilized 3ml syringes fitted with 24 gauge needle from the wing vein. Blood samples (3ml) were then transferred into pre-sterilized test tubes and serum samples were separated by centrifugation (1500 x g for 20-25 minutes). The serum was stored at –20C^0 until analysis. Blood collections were always carried out between 5:00 PM to 7:00 PM to minimize the handling stress which if not minimized can affect serum hormonal levels (Suttan *et al.*, 1973; Schanbacher *et al.*, 1973; Dessypris *et al.*, 1976; Pollard *et al.*, 1980).

EGGS COLLECTION AND HANDLING

One nest was placed in each pen in 18th week of age to accustom the birds to lay the eggs in it. Eggs were collected 3-4 times daily and stored at ambient temperature upto 3 days before being set in domestic fowl incubator. For analysis, egg production was calculated as eggs/female/week. Egg having small size or cracked were discarded. The eggs having good quality of shell and standard weight and size were selected for incubation. Eggs were rotated manually 8-10 times in 24 hours at an interval of 2-3 hours.

MORPHOMETRIC STUDY

The study consists of fixation and staining of tissues. After slaughtering the birds by Muslim method (Gracey and Collins, 1992), required tissues were removed quickly and fixed in sera for 4-5 hours. The composition of sera is given below.

Absolute alcohol	60ml
Formaldehyde	30ml
Glacial acetic acid	10

Then next the tissues were dehydrated as below in ascending grades of alcohol.

70% alcohol	3-4 hours.
80% alcohol	overnight.
90%alcohol	4 hours
100% alcohol	4 hours

After dehydration the tissues were transferred to cedar wood oil until they become clear and transparent. The tissues were then embedded in Paraplast by following procedure.

Benzol 1	10 minutes
Benzol 2	10 minutes
Benzol + Paraplast	20 minute
Paraplast 1	Morning (at 60 °C)
Paraplast 2	Evening (at 60 °C)
Paraplast 3	Morning (at60 °C)

Then these tissues were fixed on wooden blocks for cutting with the microtome. The tissues were sectioned at a thickness of 5-6um by using Reichert Microtome and stretched at 60 ^{0}C on fisher slide warmer. The prepared slides were kept on warmer at 60 °C for 24 hours so that ribbon stick on the slides. These slides were transferred to xylene 1 and xylene 2 for 15 minutes each to remove the wax. The tissues were then hydrated in the descending grades of alcohol, washed in tap water and stained in Harris Haemotoxylin. After that tissues were dehydrated with ascending grades of alcohol ,counter stained with eosin (Mcmanus and Mowry, 1960).At last slides showing different stages of oogenesis were studied.

HORMONE MEASUREMENTS

Estradiol concentration in serum was measured by using highly specific radioimmunoassay (RIA) kit (Immunotech). Samples and standards were incubated for three hours in antibody-coated tubes with an I_{125}-labled estradiol tracer. After incubation, the liquid contents of the tube were aspirated and bound radioactivity was measured by using the Gamma counter. A standard curve was established and unknown values determined by interpolation from the standard curve and following procedure was adopted for the assay.

- All the reagents were brought to room temperature prior to use.
- Anti-estradiol antibody-coated tubes were allotted the number in a single series.
- Added 100ul of standard, in tube 3-16 in duplicate series.
- Tubes 17-18 and 99-100 were quality control in which only 500ul tracer were added.
- Tubes 1 and 2 were total count without anti-estradiol antibody-coated.

- 100ul of each sample followed by 500ul of tracer was added to the remaining tubes.
- Two additional tubes added 500ul of tracer was kept in order to obtain total Cpm(T).
- The tubes were covered for overnight.
- All tubes were incubated at room temperature (18-25^{0}C) with gentle horizontal shaking (300rp) during 3 hours because correct setting of shaker is very important for the reproducibility of the assay.
- Aspirated contents of all tubes carefully except of tubes for total Cpm.
- Radioactivity of the tubes for counts bound (B) and total cpm was measured in Gamma counter.

LUTIENIZING HORMONE ASSAY

The immunoassay (immunotech) of LH is "sandwitch" type assay. The sample and standard were incubated in tubes coated with first monoclonal antibody in the presence of second antibody which is labeled with 125 I. After incubation, the contents of the tubes were aspirated and the tubes were rinsed so as to remove unbound 125 I-labled antibody. The bound radioactivity is then determined in a gamma counter. At the same time a standard curve is prepared with standards. The LH concentrations in the samples were then obtained by the interpolation from the curve. The concentration of LH in the samples is directly proportional to the radioactivity.

ASSAY PROCEDURE

Preparation of the washing solution

- Poured the contents of the vial into 1000ml of distilled water and homogenized. The diluted solution was stored at 2-8^{0}C until used.
- Prepared a series of tubes in a single series.
- Added sequentially, 100ul of standard of sample and 50ul of tracer (125_I-labled) monoclonal antibody and shaked gently.
- Prepared separately two tubes, containing 50ul of tracer only for the determination of total counts, T.
- The tubes were incubated for 90 minutes with gently shaking (350rpm) at room temp (18-25^{0}C). The correct setting of the shaker is very important for the reproducibility of the assay.
- Aspirated or decanted the contents of the tubes except of tube T.
- Washed with approximately 2ml of wash solution, aspirated. Repeated this step, so that there was no trace of the dye remained.
- Determined the radioactivity of all tubes: Counted for 2 minutes, in gamma counter with window adjusted for 125_I.

CHOLESTEROL ESTIMATION

Serum cholesterol levels were determined by enzymatic calorimetric method (ChoD-PAP Methed). The working reagent was prepared by mixing 90m. mol/1 pipes buffer

(PH 6.9), 26m. mol/l phenol, 300u/l cholesterol esterase, 300u/l cholesterol oxidase, 1250u/l peroxidase and 0.4m.mol/l of 4-aminoantipyrne, kept at 2-8^{0}C. Cholesterol solution (containing 200mg cholesterol/2ml) was used as standard. The procedure followed is described as:

	Blank	Unknown	Standard
Sample	-	20ul	-
Standard	-	-	20ul
Working reagent	2ml	2ml	20ul

Mixed and incubated at 37^{0}C for minutes and the absorbence of unknown and standard were measured against blank reagent at 505nm (500-550nm). Cholesterol levels were calculated by using the formula.

Cholesterol (m.mol/l)=Abs. of sample/Abs. of std. X Conc of std. X 0.0259.

STATISTICAL ANALYSIS

The values in tables and text are presented in Means ±SEM (number of samples). Differences were compared by analysis of variance using computer program Pad Prim 2.01 version and individuals comparisons were made by the student t-test. For the egg production analysis, weekly egg number divided by individuals in that group and tukey test was also used for the differences in weekly egg production. A Probability (P) value less than 0.05 was regarded as significant difference.

CHAPTER

3

RESULTS

EFFECT OF FURAZOLIDONE-ADMINISTRATION ON BODY WEIGHT OF THE BIRDS

The significance of furazolidone-administration and age of the birds on body weight during entire experiment (15-24 weeks of age) was determined by applying two-way ANOVA Table 2. Two-way analysis of variance shows that furazolidone-administration significantly ($F_{(3,256)}$=3.647; P=0.0132) suppressed the growth of the birds i.e body weight among the treatment groups as compared to control birds. A non-significant ($F_{(7,256)}$=0.3575; P=0.9260) difference in body weight was observed when the age of birds was compared between the control and treated groups. The treatment have the same affect on body weight with advancement in the age of birds so interaction between furazolidone-treatment and age is considered non-significant ($F_{(21,256)}$=1.042; P=0.4128).

DURING FIVE WEEK OF FURAZOLIDONE-ADMINISTRATION

Age related changes in mean body weight of control and furazolidone-fed groups during period from 15-20 week of age are shown in Table 1. The body weights were taken every week starting from 1-5 weeks of treatment. In the first week of furazolidone treatment no significant (P>0.05) effect was observed on body weight of the treated groups as compared to the control group. Whereas, furazolidone treatment during the 2nd week caused non-significant (P>0.05) decrease in the body weight of group I and group II receiving 200mg and 400mg furazolidone/kg feed per day respectively, but a significant (P<0.05) decrease was noted in the mean body weight of group III, receiving 800mg furazolidone/kg feed per day as compared to the control group. In the third week of furazolidone administration (400mg and 800mg furazolidone/kg feed) caused significant (P<0.05; P>0.02) decrease in the mean body

weight of group II and III as compared to control birds. The mean body weight of group II and group III significantly ($P<0.05$; $P<0.02$ respectively) decreased during the fourth week of furazolidone-treatment as compared to control birds. There was also a significant ($P<0.05$) decrease in the mean body weight of group III when compared to the group I. In the 5th week of furazolidone administration, a highly significant ($P<0.01$) reduction in the mean body weights of group II and group III was observed as compared to control and a significant ($P<0.05$; $P<0.01$) decrease was also noticed in the mean body weight of group II and III respectively, when compared with group I.

The results of two-way ANOVA indicate that body weight of all groups increased non-significantly ($F_{(7,128)}=0.3776$; $P=0.9140$) with increase in age of the birds. Furazolidone treatment significantly ($F_{(3,128)}=4.206$; $P=0.0071$) decreased the body weights in treated groups compared to control birds. Age interacting with treatment show highly significant ($F_{(21,128)}= 1.843$; $P= 0.0206$) effects on mean body weight indicating that treatment as an independent factor is only effective in combination with age of the birds Table 3.

AFTER TERMINATION OF FURAZOLIDONE-TREATMENT

Pattern of changes in mean body weight of control and furazolidone-treated groups after termination of furazolidone-administration in feed during the period of 21-24 week of age is presented in Table 4. In the first week of termination of furazolidone treatment, the mean body weight of group II and III significantly ($P<0.01$; $P<0.001$) decreased as compared to the control birds. Similarly, a significant ($P<0.05$; $P<0.01$) reduction in body weight was also noted when group I was compared with group II

and group III. In the second week after the termination of furazolidone treatment a significant ($P<0.02$; $P<0.001$) decrease was observed in the mean body of weight of the group II and group III as compared to control birds. The mean body weight gain of group III remained significantly ($P<0.01$) lower than the group I at 22nd week of age. In the third week after furazolidone termination, the mean body weight of group III was reduced significantly ($P<0.02$) as compared to the control group. No difference in mean body weight was noted when group II and group III were compared to group I during this week. In the fourth week after furazolidone termination, there was no significant ($P>0.05$) variation in the mean body weights of the three groups (I, II and III) as compared to the control birds.

The result of two-way analysis of variance indicates that age has no significant ($F_{(7,96)}=0.4531$; $P=0.8658$) effect on mean body weight gain of all groups. After the termination of furazolidone containing feed although the bids received furazolidone free diet, their body weight gain remained significantly ($F_{(3,96)}=5.299$; $P=0.0020$) lower in the body weights in treated groups compared to control birds. It was also observed that interaction between unmedicated feed and age of the birds was not significant ($F_{(21,96)}=0.8163$; $P=0.6937$).This indicates that, age and furazolidone free diet do not interact effectively during this period of experiment Table 5.

FEED CONVERSION RATIO

The mean values of feed conversion ratio in control and furazolidone-treated groups are given in the Table 6. During first three weeks of furazolidone exposure, there was no significant ($P>0.05$) difference in mean FCR of group I and II as compared

Table 1: Effect of oral administration of furazolidone on body weight during the period from 15-20 week of age in female Rhode Island Red birds

Treatment Groups	Ist week	2nd week	3rd week	4th week	5th week
Control	1294.63±23.20 (8)	1415.25±23.81 (8)	1511.63±21.84 (8)	1587.50±26.73 (8)	1681.25±29.07 (8)
Group-I	1261.25±36.68 (8)	1370.00±37.49 (8)	1474.38±32.75 (8)	1591.88±36.72 (8)	1665.63±31.55 (8)
Group-II	1256.88±22.49 (8)	1347.88±28.87 (8)	1425.63±32.05[a] (8)	1503.75±24.13[a] (8)	1547.38±20.85[c1] (8)
Group-III	1260.00±21.88 (8)	1331.25±23.47[a] (8)	1400.00±30.72[b] (8)	1466.63±36.05[b1] (8)	1531.25±31.46[c3] (8)

The values in the table are means ± SEM (number of birds in each group).
[a]P<0.05; [b]P<0.02; [c]P<0.01 (as compared with control).
[1]P<0.05; [3]P<0.01 (When Group I compared with Group II and Group III).

Table 2: Two-way ANOVA showing the result of effect of furazolidone-administration on body weight during period from 15-24 week of age

Source of Variation	Df	SS	MS	F	P<
Interaction	21	1424000	67820	1.042	0.4128
Treatment	3	711900	237300	3.647	0.0132
Age	7	162800	23260	0.3575	0.9260
Residual	256	16660000	65070	-	-

Table 3: Two-way ANOVA showing effect of furazolidone-treatment on body weight for five week during the period from 15-20 week of age

Source of Variation	Df	SS	MS	F	P<
Interaction	21	775500	36930	1.843	0.0206
Treatment	3	252800	84260	4.206	0.0071
Age	7	52960	7565	0.3776	0.9140
Residual	128	2565000	20040	-	-

Table: 4 Mean body weight of female Rhode Island Red birds after termination of furazolidone in feed (from 21-24 week of age)

Experimental Groups	Ist week	2nd week	3rd week	4th week
Control	1773.75±28.00 (8)	1895.63±24.67 (8)	1995.75±34.92 (8)	2066.75±46.65 (8)
Group I	1733.75±32.35 (8)	1836.25±27.02 (8)	1938.13±27.52 (8)	2015.00±14.96 (8)
Group-II	1651.38±19.98[c1] (8)	1798.75±22.34[b] (8)	1910.00±25.39 (8)	1979.00±35.57 (8)
Group-III	1573.13±26.39[d3] (8)	1724.38±23.04[d3] (8)	1879.38±19.77[b] (8)	1978.50±14.89[a] (8)

The values in the table are means ± SEM (number of birds in each group).
[a]P<0.05; [b]P<0.02; [c]P<0.01; [d]P<0.001 (as compared with control).
[1]P<0.05; [3]P<0.01 (When Group I compared with Group II and Group III).
P<0.05 (When group II and group III were compared).

Table: 5 Two-way ANOVA showing the effect of furazolidone-administration on body weight for four week of withdrawal period

Source of Variation	Df	SS	MS	F	P<
Interaction	21	395200	18820	0.8163	0.6937
Treatment	3	366500	122200	5.299	0.0020
Age	7	73120	10450	0.4531	0.8658
Residual	96	2213000	23050	-	-

Table: 6 Comparison of feed conversion ratio (FCR) in female Rhode Island Red birds fed with different levels of furazolidone in feed

Experimental Groups	1st week	2nd week	3rd week	4th week	5th week
Control	2.96±0.03 (8)	3.04±0.04 (8)	3.15±0.05 (8)	3.12±0.05 (8)	3.10±0.04 (8)
Group I	2.95±0.03 (8)	3.07±0.04 (8)	3.19±0.05 (8)	3.17±0.03 (8)	3.15±0.03 (8)
Group II	2.96±0.06 (8)	3.10±0.06 (8)	3.26±0.05 (8)	3.30±0.05[b] (8)	3.29±0.04[b1] (8)
Group III	3.02±0.06 (8)	3.19±0.06[a] (8)	3.32±0.05[a] (8)	3.29±0.05[b] (8)	3.30±0.05[b1] (8)

The values in the table are means ± SEM (number of birds in each group).
[a]P<0.05; [b]P<0.02, significantly different from control).
[1]P<0.05 (When Group I compared with Group II and Group III)

with the control group but a significant (P<0.05) decrease in FCR of group III during 2nd and 3rd week of furazolidone-treatment as compared to the control group. In 4th and 5th week of furazolidone-treatment a significant decrease (P<0.02) was observed in mean FCR values of group II and group III as compared with that of control group. Moreover, a significant (P>0.02) decrease in mean FCR of group II and group III was observed as compared to group I during this period of drug exposure.

SERUM CHOLESTEROL PROFILE

Pattern of changes in serum cholesterol concentration of control and furazolidone-treated groups during the age from 15-24 week were determined by applying two-way ANOVA and is shown in Table 8 and Figure A. The result indicates that furazolidone-treatment significantly ($F_{(3,128)}$=57.85; P=0.0001) reduced the serum cholesterol level in all treatment groups in comparison with the control group but age of the birds did not significantly ($F_{(3,128)}$=0.2714; P=0.8459) affect the serum cholesterol profile among all groups. The treatment have the same affect at serum cholesterol concentration with advancement in the age of birds so interaction is considered not significant ($F_{(9,128)}$=0.2714; P=0.6415). The weekly changes in mean serum cholesterol level during five weeks of furazolidone-administration and four weeks after termination of furazolidone are described separately as follows.

Serum cholesterol concentration during five week of furazolidone-treatment

Serum cholesterol estimated during the period from 15-20 week of age at weekly intervals in control and furazolidone-treated groups is shown in Table 7. In the first week of furazolidone-treatment, there was significant (P<0.02) decreased in mean serum cholesterol concentration of treated groups II and III as compared to control

group. Serum cholesterol level of group I did not vary ($P>0.05$) from control birds. In the second week of furazolidone-treatment the mean serum cholesterol concentration significantly ($P<0.02$) decreased in group II as compared to control birds. A significant ($P<0.01$) reduction was also observed in group III when compared with the control group. Furazolidone-treatment significantly ($P<0.05$) suppressed the serum cholesterol concentration in group III as compared to group I.

In the third week of furazolidone-administration a significant ($P<0.001$) reduction was observed in the serum cholesterol level of group II and III as compared to control birds. Furazolidone had also significantly ($P<0.001$) lowered the serum cholesterol level in group II and III as compared to group I. During the fourth week of furazolidone-medication significantly ($P<0.05$) decreased the serum cholesterol level in group I as compared to control birds. Similarly, a highly significant ($P<0.001$) decrease was also observed in serum cholesterol level of group II and group III when compared to control birds. Furazolidone had significantly ($P<0.01$; $P<0.001$) affected the serum cholesterol level in group II and group III respectively, when compared to group I. In the fifth week of experiment furazolidone-treatment significantly ($P<0.05$) lowered the serum cholesterol level of group I as compared to control birds. A highly significant ($P<0.001$) reduction was also noted in mean serum cholesterol level of group II and III when compared with control birds.

The result of two-way ANOVA indicates that the furazolidone-treatment have significant ($F_{(3,64)}=51.87$; $P=0.0001$) affected the serum cholesterol level in all treatment groups. No significant ($F_{(3,64)}=0.08301$; $P=0.4822$) effect of age on serum cholesterol level was seen. Age of the birds did not interact with treatment because treatment have the same affect at all values of serum cholesterol with increase in the

Table 7: Mean serum cholesterol concentration (m.mol/I) measured at weekly interval during furazolidone-treatment (from 15-20 week of age) in female Rhode Island Red birds

Experimental Groups	*Ist week*	*2nd week*	*3rd week*	*4th week*	*5th week*
Control	3.37±0.13 (4)	3.52±0.18 (4)	3.68±0.09 (4)	4.25±0.13 (4)	4.55±0.20 (4)
Group I	3.27±0.23 (4)	3.34±0.20 (4)	3.45±0.09 (4)	3.79±0.09[a] (4)	3.45±0.22[a] (4)
Group II	2.83±0.10[b] (4)	2.69±0.18[b] (4)	2.48±0.12[d4] (4)	2.99±0.15[d3] (4)	3.15±0.12[d] (4)
Group III	2.64±0.16[b] (4)	2.42±0.18[c1] (4)	2.37±0.06[d4] (4)	2.48±0.10[d4] (4)	2.09±0.19[d3] (4)

The values in the table are means ± SEM (number of samples).
[a]P<0.05; [b]P<0.02; [c]P<0.01; [d]P<0.001, significantly different from control.
[1]P<0.05; [3]P<0.01; [4]P<0.001 (when group I compared with group II and group III)

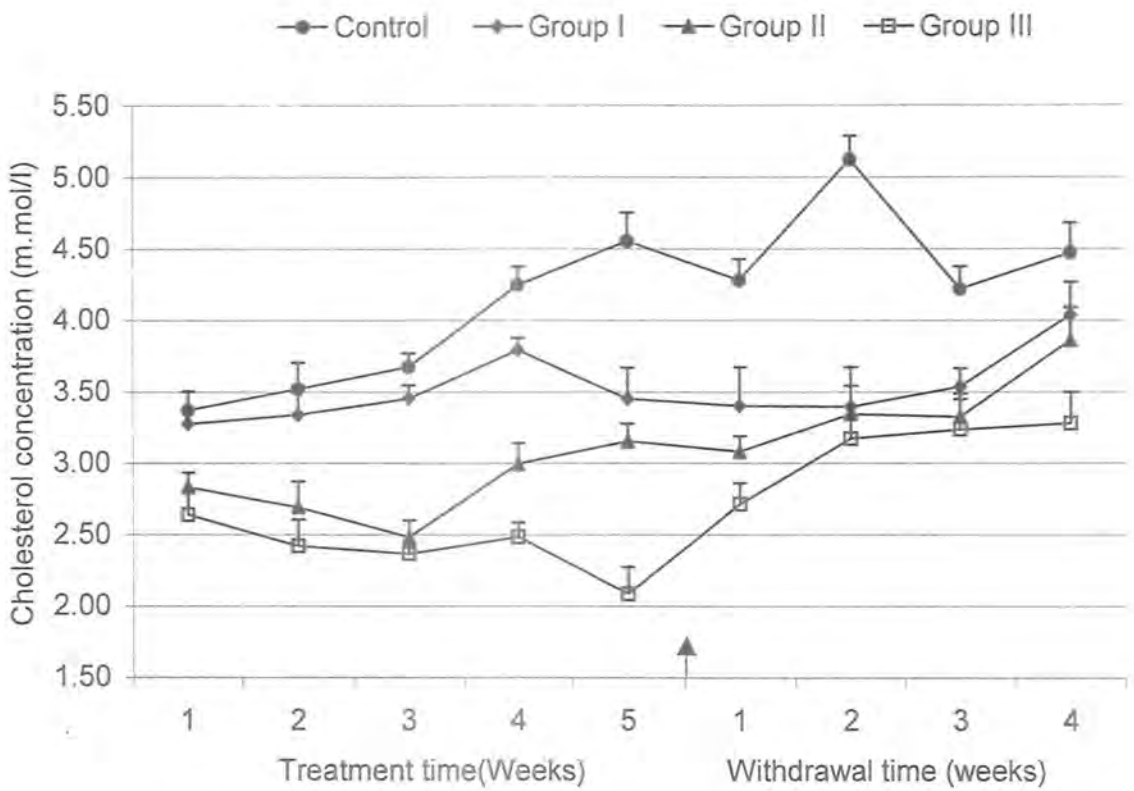


Figure A: Mean ± SEM serum cholesterol concentrations during the period from (1-5 weeks) of furazolidone treatment and (1-4weeks) after the termination of furazolidone-administration
Each point represents mean of values four birds

Table 8: Two-way ANOVA showing the effect of furazolidone-administration on serum cholesterol concentration from 15-24 week of age

Source of Variation	Df	SS	MS	F	P<
Interaction	9	1.784	0.1982	0.7730	0.6415
Treatment	3	44.51	14.84	57.85	0.0001
Age	3	0.2088	0.06960	0.2714	0.8459
Residual	128	32.83	0.2565	-	-

Table 9: Two-way ANOVA showing the effect of furazolidone-administration on serum cholesterol concentration from 15-20 week of age

Source of Variation	Df	SS	MS	F	P<
Interaction	9	1.229	0.1366	0.8267	0.5941
Treatment	3	25.71	8.569	51.87	0.0001
Age	3	0.4115	0.1372	0.8301	0.4822
Residual	64	10.57	0.1652	-	-

age so interaction was considered as non-significant ($F_{(9,64)}$=0.8267; P=0.5941) Table 9.

Mean serum cholesterol profile of four week after the withdrawal of furazolidone-treatment

Mean serum concentration of cholesterol after the withdrawal of furazolidone-treatment from 21-24 week of age is presented in Table 10. In the 21st week of age, although furazolidone-administration was ceased and furazolidone free diet was given to the birds but a significant (P<0.05) decrease was observed in the mean serum cholesterol concentration when group I was compared with control group. During the same period, the mean serum cholesterol concentration level of group II and III were significant (P<0.001) lower as compared to control birds.

While in the 22nd week of age, the mean serum cholesterol concentration still significantly (P<0.02) suppressed in group I as compared to the control birds. Similarly, there was highly significant (P<0.001) reduction in the mean serum cholesterol level of group II and III when compared with control birds.

In 23rd week of age, the mean serum cholesterol concentration significantly (P<0.05) suppressed in group I as compared to control birds. Similarly, the mean serum cholesterol level of group II and III remained significantly (P<0.01; P<0.02) lower as compared to the control birds.

At 24th week of age, the mean serum cholesterol level in group I and II approached to the control birds and no significant (P>0.05) difference was observed as compared to control birds. Whereas, the mean serum cholesterol level of group III remained significantly (P<0.02) lower than control birds.

The results of two-way ANOVA indicate that however, furazolidone-treated diet was ceased but the serum cholesterol level remained significantly ($F_{(3,48)}$=30.28; P=0.0001) lower in previously treated groups compared to control birds. A non-significant ($F_{(3,48)}$=0.8112; P=0.4940) effect on cholesterol level was observed when age of the birds was compared among all groups. The interaction between basal diet and the age of the birds was observed non-significant ($F_{(9,48)}$=0.7209; P=0.6873) Table 11.

SERUM ESTRADIOL PROFILE

Age related variation in mean serum estradiol concentrations from 15-24 weeks of age are given in Table 12 and figure B. Two-way analysis of variance was applied to find out the variation in serum estradiol level during the period from 15-24 week and also to see the changes in serum estradiol profile with increase in age of the birds Table 13. The results of two-way ANOVA indicating that during the period from 15-24 week of age furazolidone-treatment resulted in significant ($F_{(3,64)}$=11.34; P=0.0001) reduction in serum estradiol concentration in all treatment groups. Age of birds had no significant ($F_{(3,64)}$=0.09209; P=0.9641) affect on serum estradiol concentration. The treatment have the same affect at all values of estradiol level with advancement in the age of birds so there is no significant ($F_{(9,64)}$=0.1774; P=0.9958) interaction between treatment and age of the birds. The weekly changes in serum estradiol profile during the period from 15-24 week of age are elaborated below.

First Week Of Furazolidone-Treatment

In the first week of furazolidone-treatment mean serum concentration of estradiol in control group was (165.05±4.33pg/ml), in treatment group I was (157.92±5.51pg/ml),

Table: 10: Weekly mean serum cholesterol concentration (m.mol/l) after the with drawl of furazolidone-treatment from 21-24 week of age in female Rhode Island Red birds

Treatment Groups	*Ist week*	*2nd week*	*3rd week*	*4th week*
Control	4.28±0.15 (4)	5.12±0.16 (4)	4.22±0.16 (4)	4.47±0.21 (4)
Group I	3.40±0.27[a] (4)	3.39±0.28[b] (4)	3.54±0.13[a] (4)	4.04±0.23 (4)
Group II	3.08±0.11[d] (4)	3.34±0.20[d] (4)	3.32±0.16[c] (4)	3.86±0.23 (4)
Group III	2.71±0.15[d] (4)	3.17±0.16[d] (4)	3.23±0.21[b] (4)	3.28±0.22[b] (4)

The values in the table are means ± SEM (number of samples)
[a]P<0.05; [b]P<0.02; [c]P<0.01; [d]P<0.001 (as compared with control).

Table 11: Two-way ANOVA showing the effect of furazolidone-administration on serum cholesterol concentration from 21-24 week of age

Source of Variation	Df	SS	MS	F	P<
Interaction	9	1.451	0.1612	0.7209	0.6873
Treatment	3	20.31	6.771	30.28	0.0001
Age	3	0.5441	0.1814	0.8112	0.4940
Residual	48	10.73	0.2236	-	-

Table 12: Effect of furazolidone-Treatment on serum estradiol concentration (pg/ml) measured at alternate week during period from 15-24 week of age in female Rhode Island Red birds

	WITH FURAZOLIDONE-TREATMENT			WITHOUT TREATMENT	
EXPERIMENTAL GROUPS	IST WEEK (16W)	3RD WEEK (18W)	5TH WEEK (20W)	2ND WEEK (22W)	4TH WEEK (24W)
Control	165.05±04.33 (4)	181.35±04.43 (4)	196.05±03.53 (4)	201.48±04.56 (4)	194.20±04.25 (4)
Group I	157.92±05.51 (4)	181.76±06.60 (4)	193.71±04.45 (4)	190.79±04.69 (4)	186.99±03.71 (4)
Group II	154.95±05.43 (4)	160.50±05.40[a] (4)	163.45±04.28[d3] (4)	171.96±5.15[c1] (4)	184.56±3.82 (4)
Group III	150.13±05.92 (4)	157.28±05.14[b1] (4)	159.32±04.97[d3] (4)	163.11±04.45[d3] (4)	182.52±03.97 (4)

The values in the table are means ± SEM (number of birds in each group).
[a]P<0.05; [b]P<0.02; [c]P<0.01; [d]P<0.001 (as compared with control).
[1]P<0.05; [3]P<0.01 (When Group I compared with Group II and Group III).

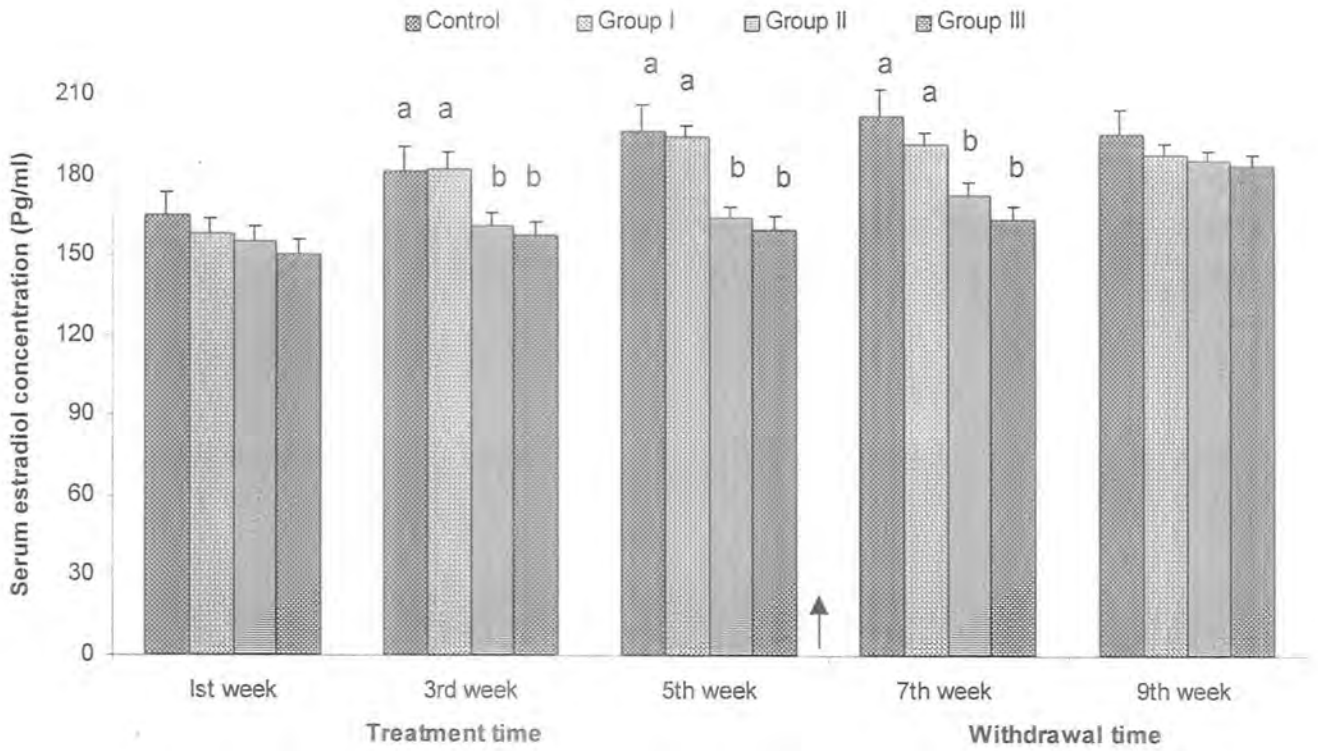


Figure B: Mean ± SEM serum estradiol concentration (Pg/ml) measured at alternate week during period from 15-24 weeks of age in female Rhode Island Red birds

↑ Start of withdrawal time.

in treatment group II (154.95±5.43pg/ml) and treatment group III was (150.13±5.92pg/ml). It was noted that during first week of furazolidone-treatment, there was no significant (P>0.05) difference in mean serum estradiol concentration of all treated groups as compared to control birds.

The results of the ANOVA indicating that in the first week, furazolidone-treatment have no significant ($F_{(3,9)}$=3.32; P=0.07) effect in estradiol concentration among the treatment groups compared to control group but a significant ($F_{(3,9)}$=6.66; P=0.01) variation was found in serum estradiol concentration within the groups Table 14.

Third Week Of Furazolidone-Treatment

In the third week of furazolidone-treatment the mean serum concentration of estradiol in control birds was (181.35±4.43pg/ml), in group I was (181.99±6.60pg/ml), in group II was (160.75±5.40pg/ml) and in group III was (157.84±5.14pg/ml). Mean serum estradiol concentration of group I was comparable (P>0.05) to the control birds. While, there was significant (P<0.05; P<0.02) decrease in mean estradiol concentration in group II and III respectively, as compared to control birds. A significant (P<0.05) reduction was also observed when group III compared with group I.

The results of two-way ANOVA shows that serum estradiol concentrations were significantly ($F_{(3,9)}$=4.57; P=0.0329) affected by furazolidone-treatment but the estradiol concentration in the groups did not vary significantly ($F_{(3,9)}$=0.14; P=0.93) Table 15.

Table 13: Two-way ANOVA showing the result of effect of furazolidone-administration on serum estradiol concentration during 15-24 week age

Source of Variation	Df	SS	MS	F	P<
Interaction	9	404.8	44.98	0.1774	0.9958
Treatment	3	8627	2876	11.34	0.0001
Age	3	70.05	23.35	0.0921	0.9641
Residual	64	16230	253.5	-	-

Table 14: Two-way ANOVA showing the result of effect of furazolidone-administration on serum estradiol concentration during first week

Source of Variation	Df	SS	MS	F	P<
Treatment	3	468.3	156.10	3.32	0.0707
Age	3	939.9	313.30	6.66	0.0116
Residual	9	423.2	47.02	-	-

Table 15: Two-way ANOVA showing the result of effect of furazolidone-administration on serum estradiol concentration in third week

Source of Variation	Df	SS	MS	F	P<
Treatment	3	2077	692.20	4.57	0.0329
Age	3	63.29	21.10	0.14	0.9339
Residual	9	1362	151.30	-	-

Fifth Week Of Furazolidone-Treatment

In the fifth week of furazolidone-administration mean estradiol concentration in control birds was (196.05±3.53pg/ml), in treatment group I was (193.71±4.45pg/ml), in treatment group II was (163.45±4.28pg/ml) and in treatment group III was (159.32±4.97pg/ml). There was no significant ($P>0.05$) difference in mean serum estradiol concentration of group I as compared to control birds. Furazolidone-administration caused a highly significant ($P<0.001$) reduction in the serum estradiol level of group II and group III as compared to control birds. Furazolidone had significantly ($P<0.01$) lowered the serum estradiol level in group II and group III when compared with group I.

The results of two-way ANOVA showing that in fifth week, furazolidone-treatment significantly ($F_{(3,9)}=17.10$; $P=0.0005$) depressed the estradiol level among the treatments groups as compared to the control group. A non-significant ($F_{(3,9)}=0.41$; $P=0.7508$) difference in estradiol concentration was found within the groups Table 16.

Second Week After Withdrawal Of Furazolidone-Treatment

In the seventh week of experiment mean estradiol concentration in control birds was (201.48±4.56pg/ml), in group I was (190.79±4.69pg/ml), in group II was (171.96±5.15pg/ml) and in group III was (163.11±4.45pg/ml). However, furazolidone free diet was fed to the birds but the mean serum estradiol concentrations remained significantly ($P<0.01$; $P<0.001$) lower in group II and III respectively, as compared to control birds. A significant ($P<0.05$; $P<0.01$) reduction was observed in the mean serum estradiol concentration of group II and group III respectively, when compared with group I.

The results of two-way ANOVA show that seventh week of experiment, although furazolidone free feed was given to birds but the estradiol serum level significantly ($F_{(3,9)}$=18.7; P=0.0003) lowered among the treatment groups as compared to the control group. Serum estradiol concentration did not alter significantly ($F_{(3,9)}$=2.45; P=0.1301) within the groups Table 17.

Fourth Week After Withdrawal Of Furazolidone-Treatment

In the ninth week of experiment the mean estradiol level in control group was observed (194.20±4.25pg/ml), in group I was (186.99±3.70pg/ml), in group II was (184.56±3.81pg/ml), and in group III was (182.52±3.97pg/ml). There was no significant (P>0.05) difference in mean serum estradiol concentration in all groups (I, II and III) as compared to control birds at this week of age.

The results of analysis of variance indicate that the serum estradiol profiles increased and became non-significantly ($F_{(3,9)}$=1.41; P=0.3034) different from control birds in all groups in this week. Similar, trend in serum estradiol concentration was observed within the groups ($F_{(3,9)}$=0.36; P=0.7820) Table 18.

LEUTEINIZING HORMONE CONCENTRATION

Mean serum luteinizing profile in the control and furazolidone treated birds during the period from 15-24 weeks of age (measured at alternate week) is presented in Table 19 and figure C. The changes in serum LH concentration during the period from 15-24 week of age and variation in serum LH concentration with increase in age of the birds were compared Table 20. The result shows that during this period of experiment, treatment, significantly ($F_{(3,64)}$=3.005; P=0.0368) reduced the serum LH level in all groups. There was no significant ($F_{(3,64)}$=0.01200; P=0.9982) effect of age on serum LH level.

Table 16: Two-way ANOVA showing the result of effect of furazolidone-administration on serum estradiol concentration in fifth week

Source of Variation	Df	SS	MS	F	p<
Treatment	3	4533	1511.00	17.10	0.0005
Age	3	108.2	36.08	0.41	0.7508
Residual	9	795	88.33	-	-

Table 17: Two-way ANOVA showing the result of effect of furazolidone-administration on serum estradiol concentration in seventh week

Source of Variation	Df	SS	MS	F	p<
Treatment	3	3656	1219	18.7	0.0003
Age	3	480.8	160.30	2.45	0.1301
Residual	9	588.2	65.36	-	-

Table 18: Two-way ANOVA showing the result of effect of furazolidone-administration on serum estradiol concentration in ninth week

Source of Variation	Df	SS	MS	F	p<
Treatment	3	311.7	103.9	1.41	0.3034
Age	3	80.26	26.75	0.36	0.7820
Residual	9	665	73.88	-	-

Table 19: Effect of furazolidone-treatment on serum Luteinizing hormone concentration (IU/L) measured at alternate week during period from 15-24 week of age in female Rhode Island Red birds.

	During FZ treatment			After FZ treatment	
Experimental Groups	Ist week (16w)	3rd week (18w)	5th week (20w)	2th week (22w)	4th week (24w)
Control	0.28±0.16 (4)	0.61±0.16 (4)	0.68±0.20 (4)	0.67±0.15 (4)	0.76±0.14 (5)
Group I	0.24±0.13 (4)	0.66±0.15 (4)	0.74±0.15 (4)	0.66±0.14 (4)	0.59±0.14[d] (4)
Group II	0.26±0.13 (4)	0.57±0.14[1] (4)	0.51±0.19[a3] (4)	0.59±0.15[a] (4)	0.55±0.16[d] (5)
Group III	0.29±0.15 (4)	0.46 ±0.22[a3] (4)	0.46±0.12[c4] (4)	0.56±0.18[a1] (4)	0.45±0.17[d] (4)

The values in the table are means ± SEM (number of samples)
[a]P<0.05; [c]P<0.01; [d]P<0.001 (as compared with control).
[1]P<0.05; [3]P<0.01; [4]P<0.001 (when Group I compared with Group II and Group III).

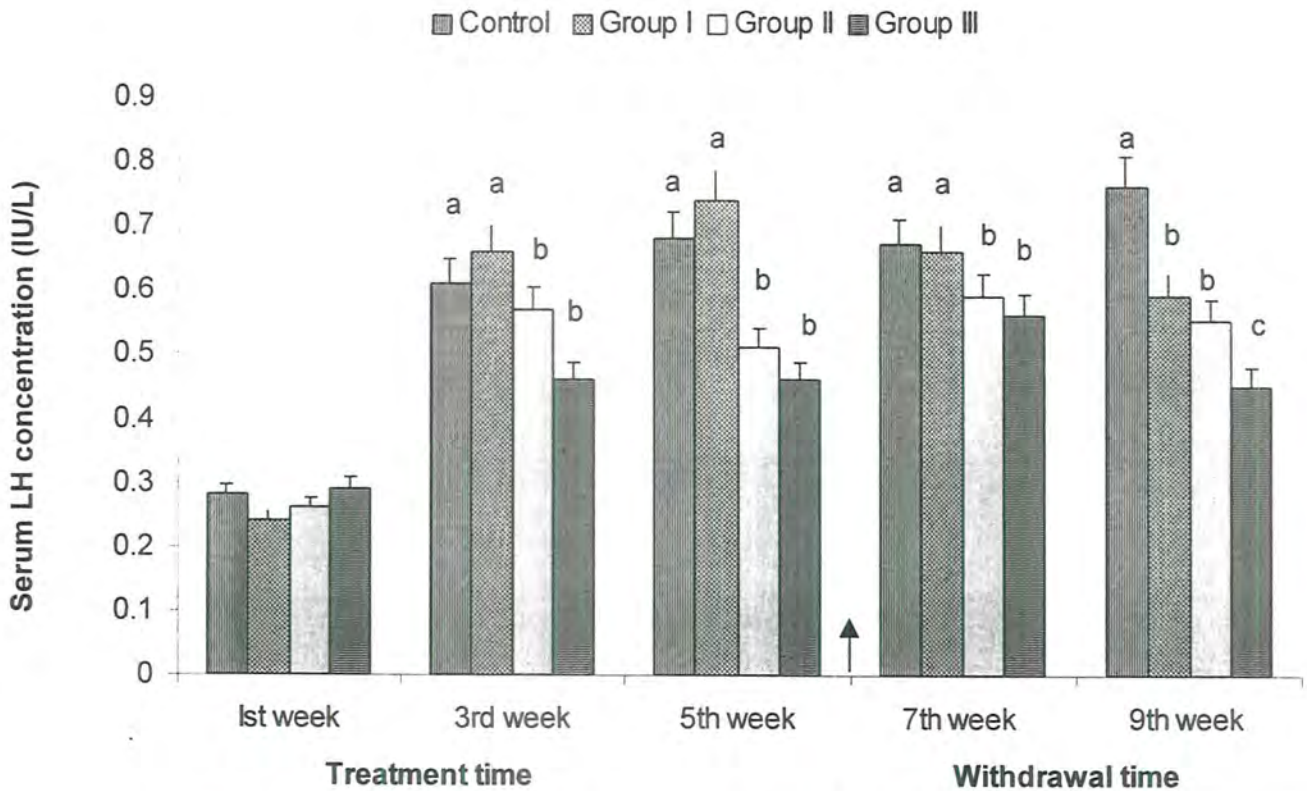


Figure C: Mean ± SEM serum LH concentration (IU/L) measured at alternate week during period from 15-24 weeks of age in female Rhode Island Red birds

↑ Start of withdrawal time.

was observed. Age does interact effectively with the treatment and treatment has the same affect on serum LH concentration with increase in the age so interaction is not significant ($F_{(9,64)}$=0.05305; P=0.10000). The variation in serum LH level during the period from 15-24 week of age are specified as follow.

First Week Of Furazolidone-Treatment

In the first week of furazolidone treatment the mean LH concentration in control group was (0.28±0.16UI/L), in treatment group I was (0.24±0.13UI/L), in treatment group II was (0.26±0.13UI/L) and in treatment group III was (0.29±0.15UI/L). There was no significant (P>0.05) difference in the mean serum LH level of all treated groups as compared to control group in this week.

The results of two-way analysis of variance indicate that there is no significant ($F_{(3,9)}$=0.775; P= 0.5368) change in LH concentration due to furazolidone-administration among treatment groups as compared to control group and non-significant ($F_{(3,9)}$= 0.503; P=0.6894), variation in LH concentration was also seen within the groups Table 21.

Third Week Of Furazolidone-Treatment

At third week of furazolidone administration the mean LH concentration in control was (0.61±0.16UI/L), in group I (0.66±0.15UI/L), in group II (0.57±0.14UI/L) and in group III (0.46±0.22UI/L). There was a significant (P<0.05) decreased in serum LH concentration of group III as compared to that of control group. Furazolidone treatment also resulted in significant (P<0.05; P<0.01) decrease in serum LH level of group II and III respectively, when compared with group I.

The result of two way ANOVA shows that furazolidone treatments have significant ($F_{(3,9)}$=5.604; P=0.019) effect on mean serum LH concentration but there was no significant ($F_{(3,9)}$=0.192; P=0.899) variation in mean serum LH concentration within the groups Table 22.

Fifth Week Of Furazolidone-Treatment

In the fifth week of furazolidone-administration mean LH concentration in control birds was (0.68±0.20UI/L), in group I was (0.74±0.15UI/L), in group II was (0.51±0.19UI/L) and in group III was (0.46±0.12UI/L). Furazolidone-administration significantly (P<0.05; P<0.01) reduced the serum LH level in group II and group III respectively, as compared to control birds. Furazolidone had also significantly (P<0.01; P<0.001) lowered the serum LH level in group II and group III as compared to group I.

The result of two-way ANOVA indicate that in fifth week, furazolidone treatment caused significant ($F_{(3,9)}$=23.41; P=0.0001) decrease in LH level among the treatments groups as compared to the control birds. A non-significant ($F_{(3,9)}$=1.624; P=0.2517) variation in LH concentration was noted when compared within the groups Table 23.

Second Week After Withdrawal Of Furazolidone-Treatment

In the second week after withdrawal of furazolidone of experiment the mean LH concentration in control group was (0.67±0.15UI/L), in group I was (0.66±0.14UI/L), in group II was (0.59±0.15UI/L) and in group III was (0.56±0.18UI/L). The serum LH concentration was significant (P<0.05) lowered in group II and Group III as compared to control group in this week. There was a significant (P<0.05) decline in mean serum LH concentration of group III when compared with group I.

The result two-way analysis of variance indicates that in this week although furazolidone-unmedicated feed was given, the mean LH serum concentration in all previously treated groups was significantly ($F_{(3,9)}$=5.449; P=0.0206) lower as compared to the control group. There was no significant ($F_{(3,9)}$=1.136; P=0.3856) difference in the LH concentration was found when concentration was compared within the groups Table 24.

Fourth Week After Withdrawal Of Furazolidone-Treatment

In the ninth week of experiment the mean LH concentration in control birds was (0.76±0.14UI/L), in group I was (0.59±0.14UI/L), in group II was (0.55±0.16UI/L) and in group III was (0.45±0.17UI/L). However, furazolidone-administration was withdrawn but in all affected birds, a highly significant (P>0.001) decrease was observed in mean serum LH level of all groups (I,II,III) when compared with control birds. No significant (P>0.05) difference in mean serum LH level was observed when all affected groups were compared with group I.

The result of two-way ANOVA indicates that even after four weeks of furazolidone withdrawal, there was significant ($F_{(3,9)}$=22.98; P=0.0001) decrease in LH concentration among the treatments groups compared to the control group and a non-significant ($F_{(3,9)}$=0.431; P=0.7362) variation in LH concentration within the groups was observed Table 25.

EGG PRODCTION

The weekly data on egg production was expressed as eggs/female/week and is shown in Figure D. Furazolidone-treatment resulted in delay egg laying in all treated groups

Table 20: Two-way ANOVA showing the result of effect of furazolidone-administration on serum LH concentration during 15-24 week age

Source of Variation	Df	SS	MS	F	P<
Interaction	9	0.01386	0.001539	0.05305	1.0000
Treatment	3	0.2616	0.08719	3.005	0.0368
Age	3	0.001045	0.0003483	0.01200	0.9982
Residual	64	1.857	0.02902	-	-

Table 21: Two-way ANOVA showing the result of effect of furazolidone-administration on serum LH concentration during first week

Source of Variation	Df	SS	MS	F	P<
Treatment	3	0.005850	0.00195	0.7748	0.5368
Age	3	0.003800	0.001267	0.5033	0.6894
Residual	9	0.02265	0.002517	-	-

Table 22: Two-way ANOVA showing the result of effect of furazolidone-administration on serum LH concentration in third week.

Source of Variation	Df	SS	MS	F	P<
Treatment	3	0.08523	0.02841	5.604	0.0191
Age	3	0.002925	0.000975	0.192	0.899
Residual	9	0.04563	0.005069	-	-

Table 23: Two-way ANOVA showing the result of effect of furazolidone-administration on serum LH concentration in fifth week

Source of Variation	Df	SS	MS	F	P<
Treatment	3	0.2203	0.07343	23.41	0.0001
Age	3	0.01528	0.005092	1.624	0.2517
Residual	9	0.02823	0.003136	-	-

Table 24: Two-way ANOVA showing the result of effect of furazolidone-administration on serum LH concentration in seventh week

Source of Variation	Df	SS	MS	F	P<
Treatment	3	0.03607	0.01202	5.449	0.0206
Age	3	0.007519	0.002506	1.136	0.3856
Residual	9	0.01986	0.002206	-	-

Table 25: Two-way ANOVA table showing the result of effect of furazolidone-administration on serum LH concentration in ninth week

Source of Variation	Df	SS	MS	F	P<
Treatment	3	0.2065	0.06884	22.98	0.0001
Age	3	0.003869	0.00129	0.431	0.7362
Residual	9	0.02696	0.002995	-	-

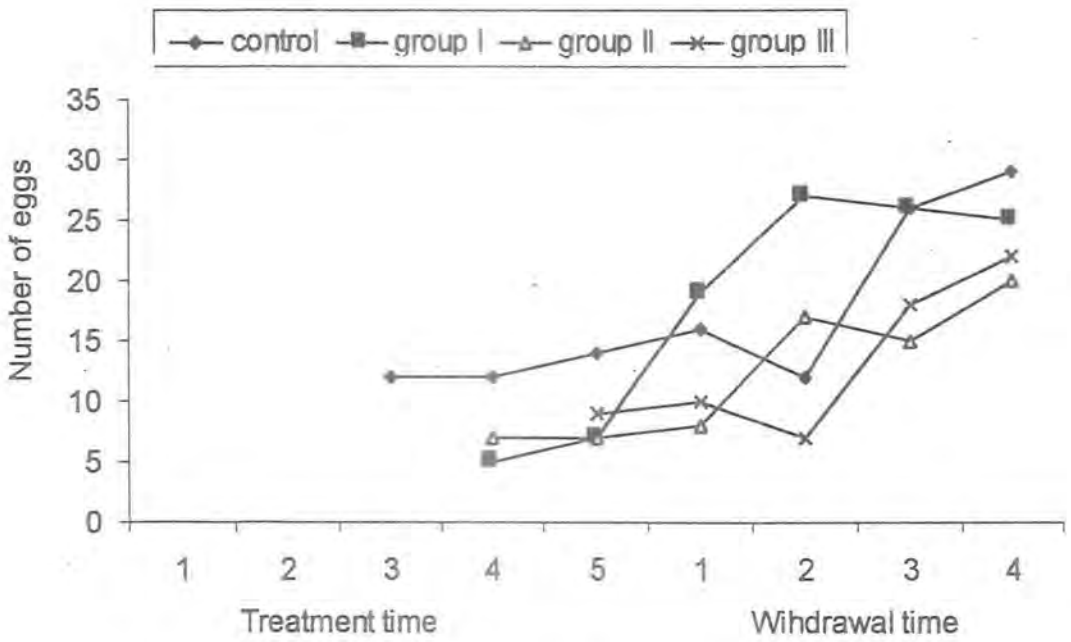


Figure 1: Weekly mean egg production (eggs/female/week) during the period treatment and withdrawal of furazolidone

which was dose dependent. In control treated groups along with increase in age, as the treatment was withdrawn that also resulted in the increase in egg production. The results with two-way ANOVA indicate that furazolidone-treatment significantly ($F_{(3,18)}$=6.686; P=0.0032) lowered the egg production among treatment groups as compared to control group. Age of the birds also significantly ($F_{(6,18)}$=16.49; P<0.0001) increased the egg production in all groups Table 26.

The result of one-way analysis of variance shows that furazolidone-treatment did not significantly ($F_{(3,27)}$=1.372; P=0.2751) affect the egg production among the treatment groups as compared to control group Table 27. On the other hand, one-way analysis of variance reveals that the age of birds resulted in significant ($F_{(6,27)}$=9.098; P<0.0001) increase in egg production Table 28. A peak in egg production was observed (P<0.05 and P<0.001) in last three week of egg laying (5-7 week). A significant (P<0.01) increase in egg production was observed when egg production of 6^{th} and 7^{th} week was compared with 2^{nd} week of egg laying. There was also significantly (P<0.05) lower number of egg production when egg production of 3^{rd} and 4^{th} week compared with 7th week egg laying.

The weekly changes in mean egg weight are presented in Table 29 . In control group significant increase in weekly mean egg weight was observed ($F_{(3,15)}$=17.41; P<0.0001) At the end of 3^{rd} week of egg laying, furazolidone-treatment was stopped. In group I, II, and III, after the withdrawal of treatment a highly significant in weekly mean egg weight gained was observed ($F_{(5,15)}$=11.66; P<0.0001) Table 30.

Furazolidone-treatment alone had non-significant ($F_{(3,27)}$=0.4110; P=0.7466) affect on the mean egg weight among all groups Table 31. There was significant ($F_{(6,27)}$=10.77; P<0.0001) increase mean egg weight with the advancement of age of the birds Table 32. A significantly (P<0.001) increase in mean eggs weight was observed in all groups with increase in age of birds.

The weekly changes in mean egg volume are shown in Table 33. In control group significant increase in weekly mean egg volume was observed ($F_{(3,12)}$=10.54; P=0.0011) At the end of 3rd week of egg laying, furazolidone-treatment was stopped. In group I, II, and III, after the withdrawal of treatment a highly significant increase in weekly mean egg volume gained was observed ($F_{(4,12)}$=7.360; P=0.0031) Table 34.

One-way analysis of variance shows the furazolidone-treatment did not significantly ($F_{(3,27)}$=0.2522; P=0.8590) alter the mean eggs volume in among all treatment groups (Table 35) when the age of birds was taken under consideration, a significant ($F_{(6,27)}$=11.42; P<0.0001) increased on mean egg volume Table 36. Mean eggs volume in all groups increased significantly (P<0.001) when compared with mean eggs volume laid during first week.

Pattern of weekly changes in mean egg size index (length/diameter) are shown in Table 37 . Furazolidone-treatment ($F_{(3,15)}$=2.887; P=0.0703) and age of the birds had non-significant ($F_{(5,15)}$=2.203; P=0.1084) affect on mean eggs size index Table 38.

The results of one-way analysis of variance show that furazolidone-treatment did not significantly ($F_{(3,27)}$=0.260; P=0.8535) affect the mean eggs size index among all treatment groups Table 39. But significant ($F_{(6,27)}$=9.447; P<0.0001) increase size of mean eggs was observed with advancement in the age of birds Table 40.

Table 26: Two-way ANOVA showing the effect of furazolidone-administration on weekly mean total egg production during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment	3	315.8	105.3	6.686	0.0032
Age	6	1558	259.6	16.49	0.0001
Residual	18	283.4	15.75		

Table 27: One-way analysis of variance showing the effect of furazolidone-administration on weekly mean total egg production during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment (between columns)	3	315.8	105.3	1.372	0.2751
Residual (within columns)	24	1841	76.71		
Total	27	2157			

Table 28: One-way analysis of variance showing the effect of age of birds on weekly mean total egg production during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment (between columns)	6	1558	259.6	9.098	0.0001
Residual (within columns)	21	599.3	28.54		
Total	27	2157			

Table 29: Effect of oral administration of furazolidone on weekly mean egg weight in female Rhode Island Red birds

Treatment time (weeks)						Withdrawal time (weeks)			
Treatment Groups	Ist week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week
Control	-	-	39.03±0.76 (7)	41.21±1.16 (7)	41.77±1.14 (7)	42.82±1.21 (7)	46.05±1.15 (7)	44.14±0.99 (7)	43.52±0.79 (7)
Group I	-	-	-	37.66±0.60 (8)	44.37±3.08 (8)	42.88±0.66 (8)	44.79±0.50 (8)	48.42±1.52 (8)	45.81±0.58 (8) (8)
Group II	-	-	-	32.88±0.79 (8)	39.36±1.71 (8)	38.32±0.21 (8)	42.08±1.40 (8)	43.38±0.69 (8)	44.62±1.02 (8)
Group III	-	-	-	-	47.45±2.28 (8)	44.36±1.68 (8)	48.68±0.89 (8)	49.51±0.84 (8)	48.34±1.28 (8)

The values in table are mean ± SEM (number of samples)

Table 30: Two-way ANOVA showing the effect of furazolidone-administration on weekly mean egg weight during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment	3	143.6	47.87	17.41	0.0001
Age	5	160.3	32.06	11.66	0.0001
Residual	15	41.24	2.749	-	-

Table 31: One-way analysis of variance showing the effect of furazolidone-administration on weekly mean weight eggs during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment (between columns)	3	264.5	88.17	0.4110	0.7466
Residual (within columns)	24	5148	214.5	-	-
Total	27	5413	-	-	-

Table 32: One-way analysis of variance showing the effect of age of birds on weekly mean eggs weight during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment (between columns)	6	4085	680.8	10.70	0.0001
Residual (within columns)	21	1328	63.24	-	-
Total	27	5413	-	-	-

Table 33: Effect of oral administration of furazolidone on weekly mean egg volume in female Rhode Island Red birds

	Treatment time (weeks)					Withdrawal time (weeks)			
Treatment Groups	Ist week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week
Control	-	-	36.45±1.00 (7)	38.17±1.29 (7)	38.47±1.04 (7)	39.73±1.49 (7)	44.50±1.56 (7)	41.40±1.13 (7)	40.77±0.75 (7)
Group I	-	-	-	36.67±1.29 (8)	40.14±3.53 (8)	39.61±0.84 (8)	41.44±0.62 (8)	45.72±1.51 (8)	43.56±0.80 (8)
Group II	-	-	-	39.22±1.19 (8)	39.00±2.33 (8) (8)	35.14±0.14 (8)	40.20±1.47 (8)	40.11±0.88 (8)	41.65±1.13 (8)
Group III	-	-	-	-	42.13±2.97 (8)	41.40±1.55 (8)	47.40±1.42 (8)	45.47±0.85 (8)	46.47±1.16 (8)

The values in the table are mean ± SEM (number of samples)

Table 34: Two-way ANOVA showing the effect of furazolidone-administration on weekly mean egg volume during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment	3	75.63	25.21	10.54	0.0011
Age	4	70.39	17.60	7.360	0.0031
Residual	12	28.69	2.391	-	-

Table 35: One-way analysis of variance showing the effect of furazolidone-administration on weekly mean volume eggs during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment (between columns)	3	144.4	48.12	0.2522	0.8590
Residual (within columns)	24	4580	190.8	-	-
Total	27	4724	-	-	-

Table 36: One-way analysis of variance showing the effect of age of birds on weekly mean eggs volume during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment (between columns)	6	3616	602.6	11.42	0.0001
Residual (within columns)	21	1109	52.79	-	-
Total	27	4724	-	-	-

Table 37: Effect of oral administration of furazolidone on weekly mean egg size index in female Rhode Island Red birds

Treatment time (weeks)					Withdrawal time (weeks)				
Treatment Groups	Ist week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week
Control	-	-	1.32±0.01 (7)	1.32±0.02 (7)	1.32±0.02 (7)	1.35±0.03 (7)	1.34±0.02 (7)	1.33±0.01 (7)	1.33±0.01 (7)
Group I	-	-	-	1.31±0.02 (8)	1.39±0.04 (8)	1.34±0.01 (8)	1.37±0.02 (8)	1.34±0.01 (8)	1.32±0.02 (8)
Group II	-	-	-	1.34±0.02 (8)	1.40±0.02 (8)	1.41±0.02 (8)	1.38±0.01 (8)	1.34±0.01 (8)	1.34±0.01 (8)
Group III	-	-	-	-	1.34±0.02 (8)	1.38±0.03 (8)	1.32±0.02 (8)	1.34±0.01 (8)	1.35±0.02 (8)

The values in the table are mean ± SEM (number of samples)

Table 38: Two-way ANOVA showing the effect of furazolidone-administration on weekly mean egg size index during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment	3	0.004146	0.001382	2.887	0.0703
Age	5	0.005271	0.001054	2.203	0.1084
Residual	15	0.007179	0.0004786	-	-

Table 39: One-way analysis of variance showing the effect of furazolidone-administration on weekly mean eggs size index during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment (between columns)	3	0.1535	0.05116	0.2600	0.8535
Residual (within columns)	24	4.722	0.1968	-	-
Total	27	4.876	-	-	-

Table 40: One-way analysis of variance showing the effect of age of birds on weekly mean eggs size index during whole experiment

Source of Variation	Df	SS	MS	F	P<
Treatment (between columns)	6	3.558	0.5930	9.447	0.0001
Residual (within columns)	21	1.318	0.06277	-	-
Total	27	4.876	-	-	-

OVARIAN WEIGHT, SIZE AND VOLUME

Mean ovarian weight and volume in the control and all furazolidone-administered groups are presented in Table 41. The mean ovarian weight (ovary with yolky follicles) in the groups fed different levels of furazolidone were similar to that of control group. A dose of 200mg/kg feed per day had no significant ($P>0.05$) effect on the mean ovarian weight (ovary without yolky follicle) as compared to control group. However, furazolidone-administration significantly ($P<0.01$) decreased the ovarian weight (ovary without yolky follicles) in the group II and group III as compared with that of control group. Moreover, a significant ($P<0.05$) decrease in mean ovarian weight was also obtained in group III given 800mg furazolidone/kg feed per day when compared with the group I fed 200mg furazolidone/kg feed per day.

Mean ovarian size or breadth (length and width) of furazolidone-fed groups showed no significant ($P>0.05$) difference all treated groups as compared with that of control group, except in group II where the mean ovarian width significantly ($P>0.05$) decreased as compared to control birds.

The furazolidone administration caused significant ($P<0.02$) reduction in mean ovarian volume (without yolky follicles) of group II as compared with control group. A significant ($P<0.01$) decrease in mean ovarian volume was also observed in group III as compared with that of control group. However, the treatment of various doses of furazolidone caused no significant ($P>0.05$) difference in the volume of ovary with yolky follicles as compared to control group.

Table 41: Effect of oral administration of furazolidone on ovarian weight and volume in female Rhode Island Red birds

Experimental Groups	Ovarian Weight (gm)		Ovarian size (cm)		Ovarian Volume (cm^3)	
	With large Follicles	Without large follicles	Length	Width	With large follicles	Without large follicles
Control	44.13±8.17 (7)	4.28±0.31 (7)	2.81±0.13 (7)	2.13±0.09 (7)	46.63±7.54 (7)	4.28±0.31 (7)
Group I	45.22±2.73 (8)	3.80±2.07 (8)	2.67±0.12 (8)	2.17±0.14 (8)	46.43±2.99 (8)	3.49±0.47 (8)
Group II	47.65±3.77 (7)	3.22±0.18[c] (7)	2.46±0.07 (7)	2.06±0.38 (7)	48.71±4.37 (7)	3.17±0.26[b] (7)
Group III	42.64±2.65 (7)	3.01±0.25[c*] (7)	2.58±0.22 (7)	2.00±0.16 (7)	40.83±2.80 (7)	2.92±0.16[c] (7)

The values in the table are means ± SEM (number of birds in each group).
[a]P<0.05; [b]P<0.02; [c]P<0.01 (as compared with control).
* P<0.05 (When Group I compared with Group II and Group III).

OVIDUCT

The mean oviduct weight of control and furazolidone-treated groups are given in Table 42. The mean weight of oviducts of all furazolidone-treated groups showed no significant ($P>0.05$) difference as compared to the control group. There was no significant ($P>0.05$) difference in the mean length of oviduct in group I fed 200mg furazolidone/kg feed per day as compared to control birds. However, in group II and III treatment with 400mg and 800mg furazolidone/kg feed per day caused in a significant ($P<0.05$ and $P<0.001$ respectively), decrease in the mean oviduct length compared with the control group. A significant ($P<0.02$) decrease was also observed in mean oviduct length of group III as to compared group I. But different doses of furazolidone administration in various groups had no significant ($P>0.05$) effect on mean oviduct volume.

COMBS AND WATTLES

Effect of different doses of furazolidone on development of secondary sexual organs measured in terms of length, width and weight of combs and wattles are shown in Table 44. The mean length and width of combs in furazolidone fed groups showed no significant ($P>0.05$) difference as compared with the control group. Similarly, the difference in mean length and width of both right and left wattles were not significantly ($P>0.05$) compared with that of control group. The mean weight of combs and wattles of furazolidone-treated groups were also showed no significant ($P>0.05$) change compared with that of control birds.

Table 42: Effect of oral administration of furazolidone on oviduct weight, length and volume of female Rhode Island Red birds.

	Oviduct		
Experimental Groups	Weight (gm)	Length (cm)	Volume (cm^3)
Control	50.38±2 (7)	63.50±2 (7)	51.57±8 (7)
Group I	48.74±2 (8)	59.06±3 (8)	45.50±4 (8)
Group II	47.50±2 (7)	55.00±2[a] (7)	43.14±2 (7)
Group III	47.34±5 (8)	47.50±3[2c] * (8)	42.25±2 (8)

The values in the table are means ± SEM (number of samples).
[a]P<0.05; [c]P<0.01 (as compared with control).
* [2]P<0.02 (When Group I compared with Group II and Group III).

Table 43: Effect of furazolidone-administration on liver weight

Treatment groups	Liver weight (gram)
Control	49.70±7
Group I	54.24±3
Group II	46.05±4
Group III	47.72±2

The values in the table are mean ± SEM (number of samples)

Table 44: Effect of different doses of furazolidone administration on development of secondary sexual organs in female Rhode Island Red birds

Treatment Groups	Comb		Right wattle		Left wattle		Comb	Wattle
	Length (cm)	Width (cm)	Length] (cm)	Width (cm)	Length (cm)	Width (cm)	weight (gm)	weight (gm)
Control	5.19±0.67 (7)	2.53±0.45 (7)	2.27±0.45 (7)	2.66±0.50 (7)	2.11±0.41 (7)	2.64±0.49 (7)	3.43±0.89 (7)	1.79±0.50 (7)
Group I	4.77±0.68 (8)	2.43±0.50 (8)	2.10±0.33 (8)	2.33±0.40 (8)	2.07±0.32 (8)	2.37±0.42 (8)	2.80±0.94 (8)	1.43±0.55 (8)
Group II	4.62±0.51 (7)	2.58±0.43 (7)	1.85±0.42 (7)	2.12±0.34 (7)	1.92±0.36 (7)	2.20±0.36 (7)	2.70±0.55 (7)	1.30±0.39 (7)
Group III	4.37±0.57 (8)	2.26±0.48 (8)	2.07±0.42 (8)	2.17±0.44 (8)	2.01±0.39 (8)	2.13±0.41 (8)	2.41±0.61 (8)	1.64±0.53 (8)

The values in the table are means ± SEM (number of birds in each group).
No significant difference was observed among the mean value

LIVER

In furazolidone-treated groups of female RIR birds, the liver appeared light brownish in colour. Consistency of the liver was friable in the groups fed with 400mg and 800mg of furazolidone/Kg feed per day for 5 weeks. While there was no alteration in the consistency of liver of the group given 200mg of furazolidone/Kg feed per day for a period of 5 week.

Mean liver weights of control and furazolidone-treated groups are presented in Table 43. There was no significant ($P>0.05$) difference in the mean liver weights of furazolidone-administered groups as compared with the control group.

OVARIAN FOLLICLES

Effect of oral administration of furazolidone at different doses on oocyte diameter is shown in Table 45. Mean number of oocyte of different sizes ranged from 1-800μm in control group birds whereas 1-700μm in group I and 1-600μm in group II and III were observed. The oocytes diameter was divided into eight different categories with an interval of 100μm. The number of oocyte in the range of 1-100μm diameter showed non-significant ($P>0.05$) difference in all groups compared to control birds. However, a significant ($P<0.05$; $P<0.02$; and $P<0.001$) decrease in mean number of oocyte in the category ranging from 101-200μm was observed in group I, II and III respectively, compared to control birds. Highest decrease was observed in group given higher dose. The mean number of oocyte sizes between 201-300μm showed significant ($P<0.05$; $P<0.001$) reduction in group I, II and III compared to control group. The mean number of oocyte in the range of 301-400μm diameter showed

significant (P<0.05; P<0.01) decrease in group I, II and III as compared to control birds. When the mean number of the oocyte in the range of 401-500μm diameter were compared, a significant (P<0.001) decrease was noted in group III compared to control birds. While, group I and II did not vary significantly (P>0.05) from control birds. Furazolidone-administration also significantly (P<0.02; P<0.01) decreased the mean number of oocyte in the range of 501-600μm diameter in group II and III respectively. The mean numbers of oocytes in range of 601-700μm diameter were not found in the group II and III. In group I oocytes in the range of 601-700μm were present but did not significantly (P>0.05) different from control birds. No oocyte in the range of 701-800μm diameter in all treated groups was observed.

The follicles were surrounded by granulosa layer of variable thickness. Mean numbers of follicle categorized according to the thickness of granulosa layer are shown in Table 46. In category 1-10μm follicular thickness the decrease in mean number of follicles was significant (P<0.05), in group I and II but highly significant (<0.001) difference was seen in group III compared to control group. In the remaining categories of follicular epithelium in group I, II and III the mean number of follicles was not significantly (P>0.05) different from control group.

The mean number of oocyte nuclei in control and treated birds is presented in Table 47. The ovarian mean number of oocyte nuclei diameter measurement data were graded as 1-20μm, 21-40μm, 41-60μm and 61-80μm in control and treated groups. The mean number of oocyte nuclei in the range of 1-20μm showed non-significant (P>0.05)

Table 45: Mean number and size gradation of follicle in control and furazolidone treated birds at 24 week of age

Treatment groups	Follicular diameter range (μm)							
	1-100μm	101-200μm	201-300μm	301-400μm	401-500μm	501-600μm	601-700μm	700-800μm
Control	12.31±1.57 (7)	10.60±1.10 (7)	9.70±0.94 (7)	6.65±0.86 (7)	3.70±0.53 (7)	3.50±0.48 (7)	3.40±0.59 (7)	3.00±0.35 (7)
Group I	8.93±1.37 (8)	6.75±0.99[a] (8)	6.35±1.05[a] (8)	4.20±0.64[a] (8)	2.47±0.41 (8)	2.47±0.60 (8)	2.33±0.25	-
Group II	14.39±2.24 (8)	6.40±0.78[b] (8)	3.89±0.45[d] (8)	2.67±0.44[b] (8)	2.21±0.48 (8)	1.75±0.31[b] (8)	-	-
Group III	9.63±1.39 (8)	4.55±0.60[d] (8)	3.95±0.58[d] (8)	2.53±0.41[b] (8)	1.33±0.23[c] (8)	1.36±0.17[c] (8)	-	-

The values in the table are mean ± SEM (number of samples)
[a]P<0.05; [b]P<0.02; [c]P<0.01; [d]P<0.001 (as compared with control)

Table 46: Mean number of follicles and thickness of granulosa cells layer of in control and furazolidone treated birds at 24 week of age

Treatment groups	Granulosa cells layer thickness diameter range (μm)				
	1-10μm	11-20μm	21-30μm	31-40μm	41-50μm
Control	12.54±2.65 (7)	9.36±3.54 (7)	7.45±3.12 (7)	4.23±1.24 (7)	3.56±0.88 (7)
Group I	10.56±3.59[a] (8)	7.26±0.98 (8)	5.63±2.36 (8)	3.47±1.4 (8)	2.89±0.84 (8)
Group II	9.4±2.37[a] (8)	8.69±1.83 (8)	6.54±3.45 (8)	4.65±1.26 (8)	3.7±0.64 (8)
Group III	8.36±1.22[d] (8)	8.35±3.57 (8)	7.49±2.22 (8)	5.69±1.33 (8)	1.33±1.93 (8)

The values in the table are mean ± SEM (number of samples)
[a]P<0.05; [d]P<0.001 (as compared with control group)

Table 47: Mean number of oocytes and size gradation of follicular nuclei in control and furazolidone treated birds at 24 week of age

Treatment groups	Oocytes nuclei diameter range (μm)			
	1-20μm	21-40μm	41-60μm	61-80μm
Control	2.67±0.74 (7)	7.25±0.83 (7)	5.67±0.41 (7)	1.40±0.19 (7)
Group I	3.00±0.93 (8)	4.75±0.78 (8)	3.00±0.71[b] (8)	-
Group II	1.33±0.20 (8)	3.67±0.89[a] (8)	2.71±0.70[b] (8)	-
Group III	1.00±0.00 (8)	2.71±0.60[c] (8)	2.50±0.35[d] (8)	-

The values in the table are mean ± SEM (number of samples)
[a]P<0.05; [b]P<0.02; [c]P<0.01; [d]P<0.001; [d]P<0.001 (as compared with control group)

number of oocyte nuclei in the range of 1-20μm showed non-significant (P>0.05) difference in all treated groups compared to control birds. A significant (<0.05; <0.01) decrease in mean number of oocyte in the range of 21-40μm diameter in group II and III but in group I this difference compared to control was not significant (P>0.05). When mean number of oocyte nuclei in the range of 41-60μm were compared with control birds, a significant (P<0.01; P<0.001) decrease was observed in group I, II and III. The oocyte nuclei having 60-80μm diameter were not observed in all treated groups.

The yolky ovarian follicles examined in this study were divided on the basis of their diameter into following categories: 1-5mm, 6-10mm, 11-15mm, 16-20mm, 21-25mm and 26-30mm. The smallest detectable follicle was 1mm and the largest was 30mm Table 48.The mean number of yolky follicles in the range of 1-5mm were greater in all groups. There was a steady decline in the mean number of follicles with increase in the yolky follicular diameter. A significant (P<0.01; P>0.05) decrease in the mean number of follicles in the diameter range of 1-5mm was observed in group I, II and III. Furazolidone-administration also significantly (P<0.01; P<0.05) decreased the mean number of yolky follicles in the diameter range of 6-10mm in group I, II and III compared to control birds. When mean number of yolky follicles of diameter range from 11 to 15mm and 16-20mm were compared with control a non-significant (P>0.05) reduction in mean number of yolky follicles in all treated groups was observed. The mean number of yolky follicles in the diameter range of 21-25mm showed highly significant (P<0.001; P<0.05) reduction in number in group I, II and III compared to control birds. The mean numbers of yolky follicles of diameter range from 26 to 30mm were comparable in control and treated groups.

Table 48: Mean number and size gradation of yolky follicles in control and furazolidone treated birds at 24 week of age

Treatment groups	Yolky follicular diameter range (mm)					
	1-5mm	6-10mm	11-15mm	16-20mm	21-25mm	26-30mm
Control	19.6±8.82 (7)	11.13±4.18 (7)	1.40±0.24 (7)	1.75±0.43 (7)	3.33±0.26 (7)	2.50±0.40 (7)
Group I	7.50±4.00[c] (8)	6.75±0.92[a] (8)	1.50±0.26 (8)	1.75±0.22 (8)	1.50±0.24[d] (8)	1.50±0.26 (8)
Group II	10.33±4.22[a] (8)	4.88±2.39[b] (8)	1.80±0.37 (8)	1.17±0.18 (8)	1.71±0.34[a] (8)	1.71±0.42 (8)
Group III	9.00±3.97[a] (8)	6.60±2.46[a] (8)	1.29±0.34 (8)	1.67±0.52 (8)	1.43±0.35[d] (8)	1.86±0.54 (8)

The values in the table are mean ± SEM (number of samples)
[a]P<0.05; [b]P<0.02; [c]P<0.01; [d]P<0.001 (as compared with control group)

The cross section of the middle portion of the ovary of control and treated Rhode Island Red birds, showed different stages of developing follicles of variable sizes in the range of 1-800μm in control group and 1-600μm in treated birds.

Furazolidone-treatment induced various change in organization of ovarian structures. The follicles of the control group showed nucleus with ooplasma, single cuboidal cell layer of granulosa , distinct basal lamina and theca interna and externa. Well organized stromal tissue were also observed Figure 1a. Where as in treated birds thecal layers were thin and basal lamina was not well distinguished. There was no clear demarcation between granulosa and thecal layer Figure 1b. Furazolidone–treatment showed various changes in the stromal tissue in treated birds. The stromal tissue in the control was compact and well vascularized but in case of treated birds it was loosely organized Figure 2abc. In the thecal intera abundant thecal glands were observed in control birds Figure 3a. But in case of treated birds no such glands were found Figure 3b.

Oocyte of the control follicle contained distinct nucleus with clear nuclear membrane, with chromatin bodies in the nucleus, were also present in the ooplasma Figure 4a. where as in treated birds disrupted nucleus was observed in the group given higher doses of furazolidone Figure 4bc.

The granulosa layer of control follicles were well organized and uniform. Darkly and lightly stained cells were also seen in the granulosa layer. Well defined Cytoplasmic extension, originating from granulosa layer penetrating the zona pellucida were observed. In control birds conspicuous zona pellucida and yolky bodies were examined Figure 5a. But in treated birds zona pellucida showed degenerative changes.

In treated birds granular layer was disrupted layer and disorganized. Darkly stained bodies were abundant. Vacuolated regions were also present between granulosa cell layer and interstitium Figure 5bc. Some atretic follicles were also observed in the treated bird. These atretic follicles were distinguished by their deformed appearance and indiscriminated granulosa and thecal cell layer. There was no demarcation between these cell layers Figure 6. In control birds larger and less smaller size follicles on the cortical region. Whereas in the treated birds comparatively smaller size follicles were examined Figure 7abc.

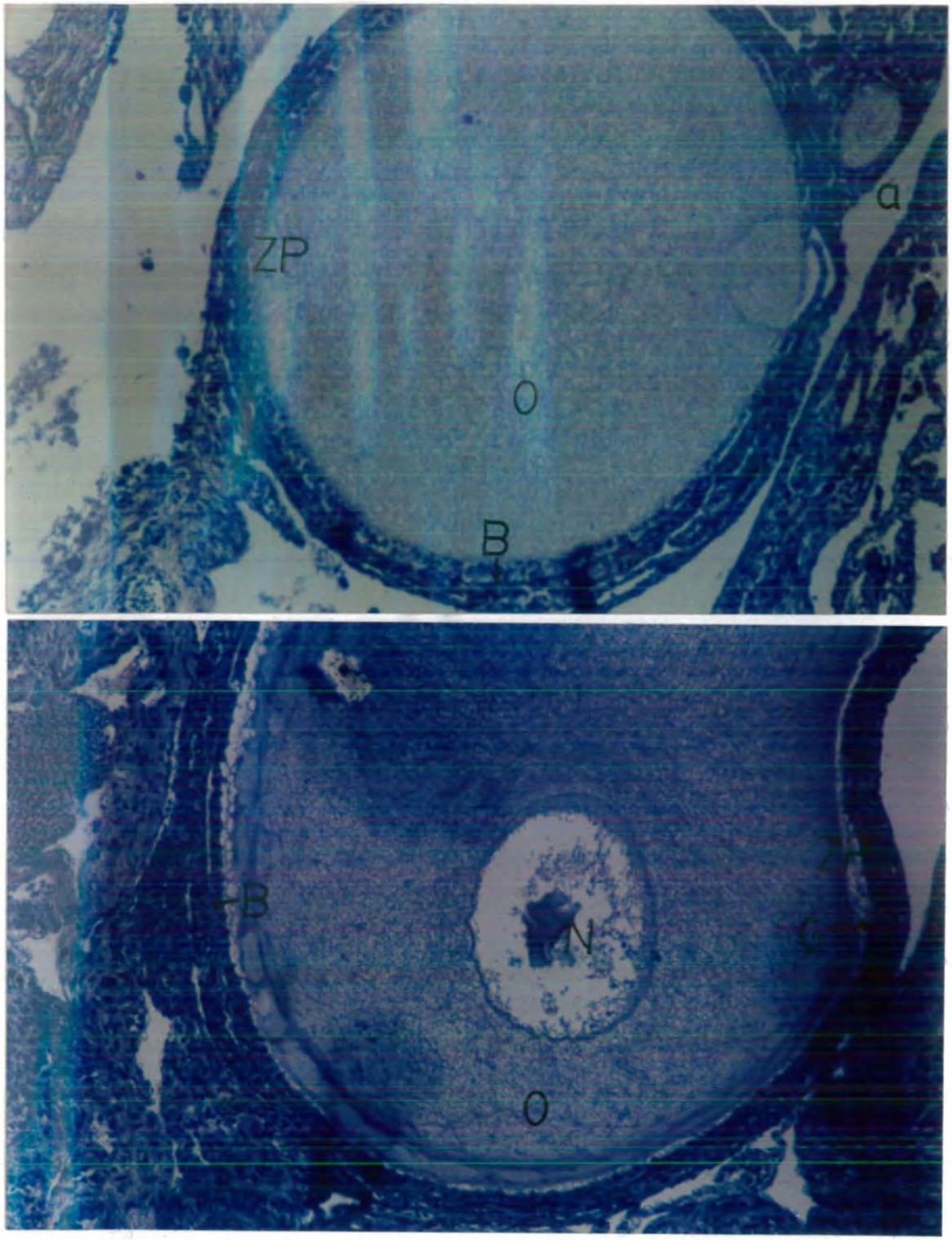


Figure 1: Photomicrographs of the middle portion of ovary of the Rhode Island Red bird at 24 weeks of age. a) Control ovary follicle showing theca interna and externa (T) zona pellucida (ZP) ooplasm (O) basal lamina (B) and granulosa (G). b) Showing follicles of treated birds thecal (T) degenerated zona pellucida (ZP) ooplasm (O) vacuolated basal lamina (B) and granulosa (G). b) and nuleus. (H.E) ×480

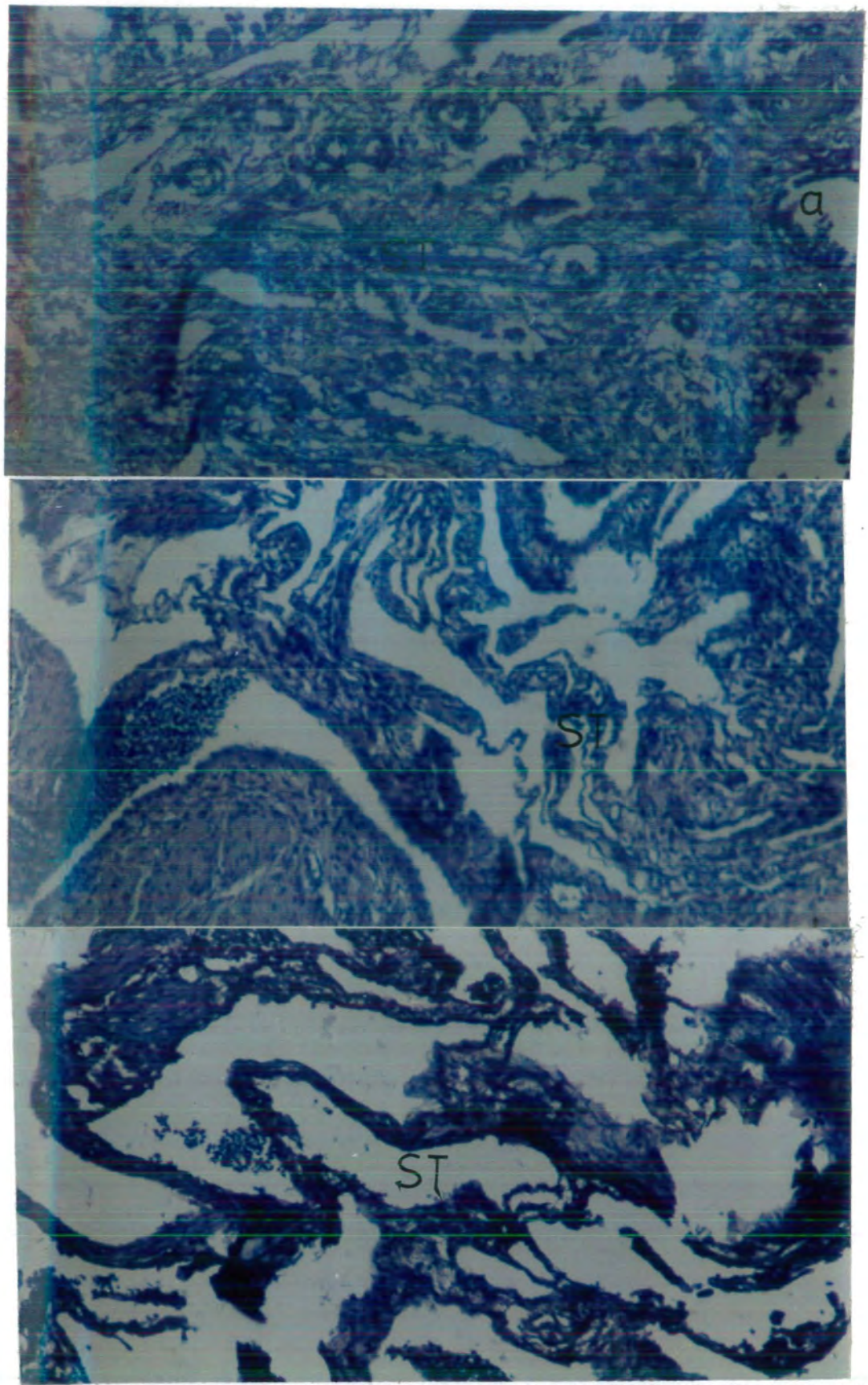


Figure 2: Photomicrographs of the middle portion of ovary of the Rhode Island Red bird at 24 weeks of age. a) Control ovary showing compactly arranged stromal tissue (ST). b, c) In treated groups stromal tissue are loosely arranged.(ST). (H.E) ×480

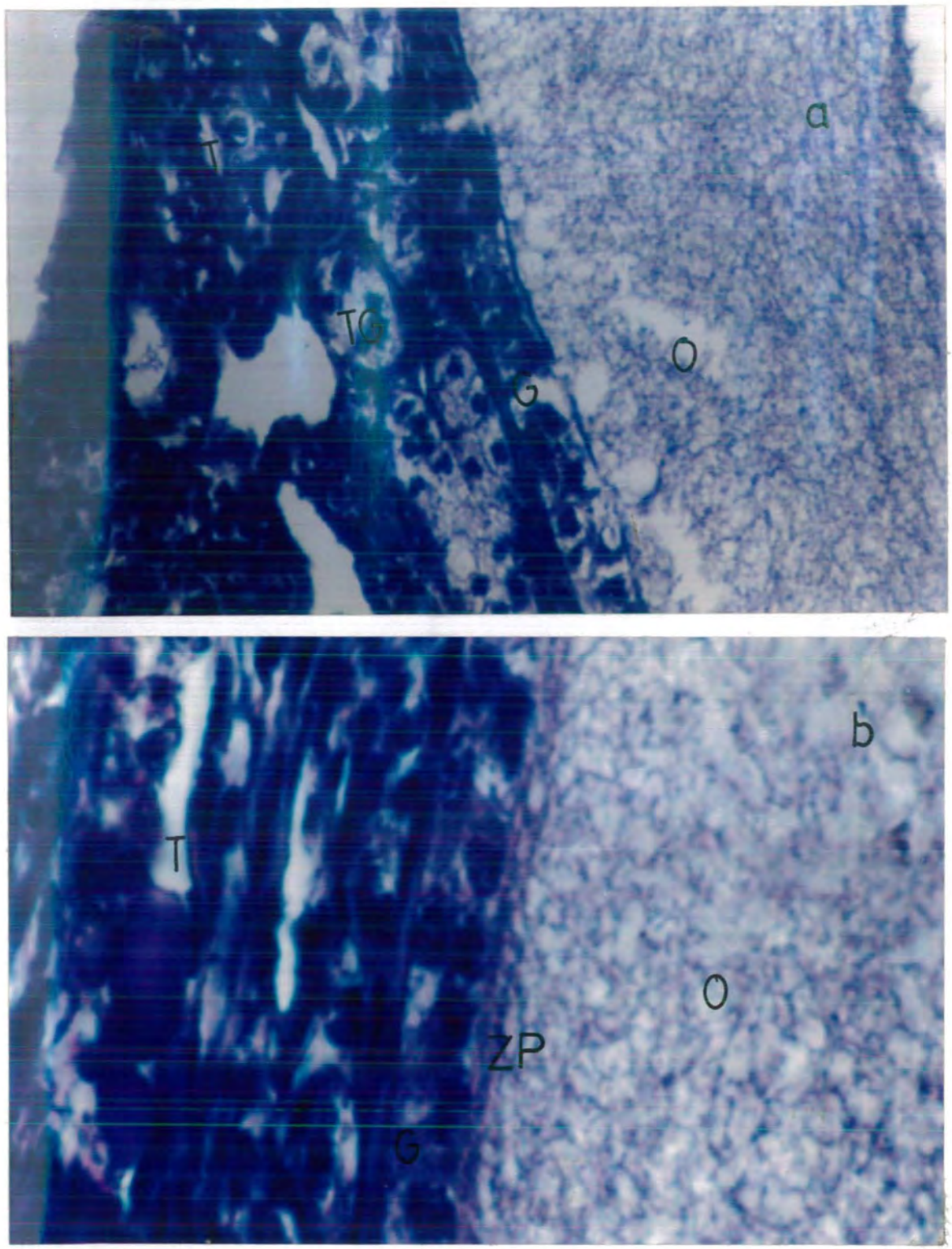


Figure 3: Photomicrographs of the middle portion of ovary of the Rhode Island Red bird at 24 weeks of age. a) Control ovary follicle showing abundant thecal gland in developing follicle (TG). B) Thecal glands are not seen in all treated groups. (H.E) ×960

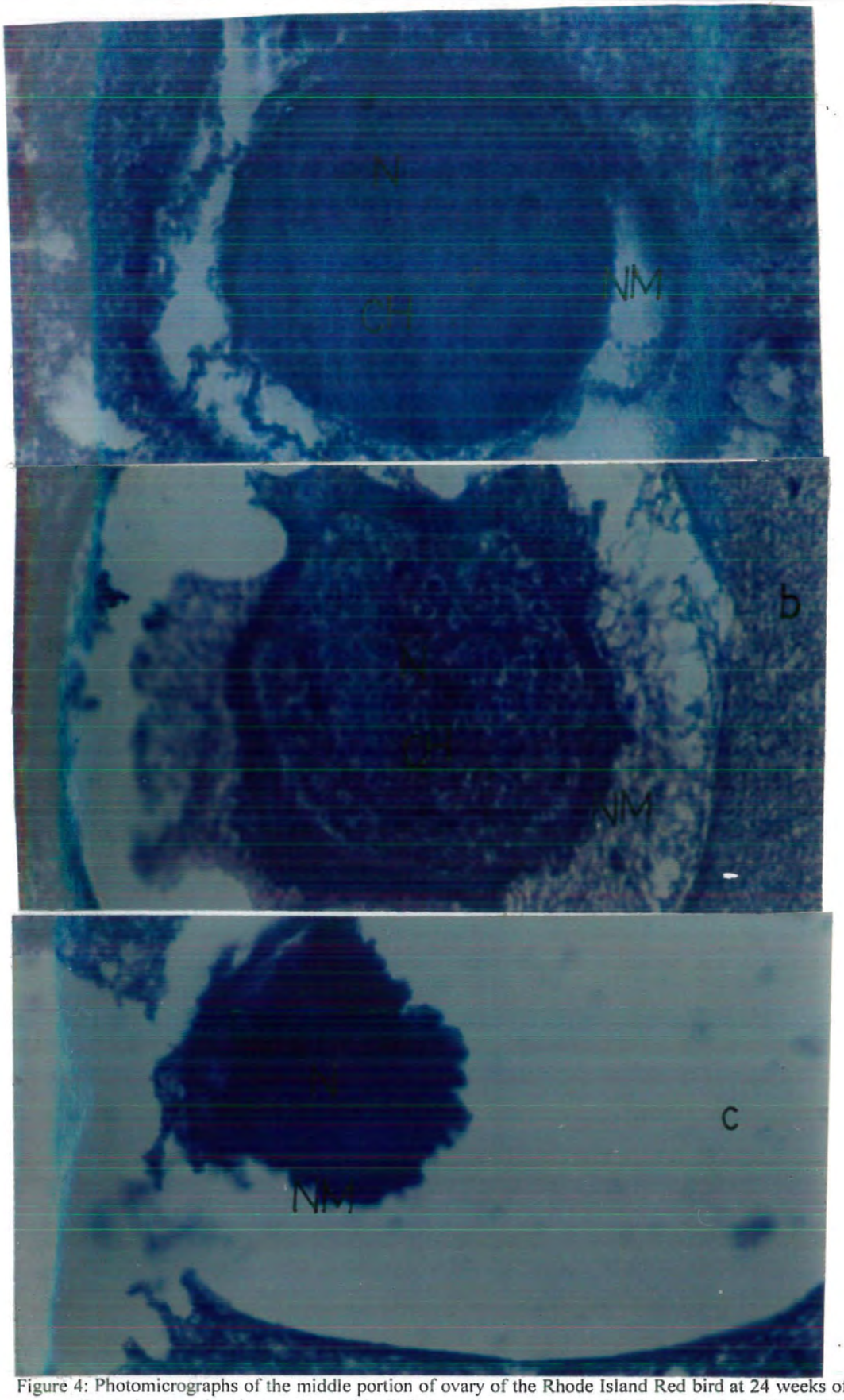


Figure 4: Photomicrographs of the middle portion of ovary of the Rhode Island Red bird at 24 weeks of age. a) Control ovary showing nucleus with distinct nuclear membrane (NM) chromatin bodies (CH). b, c) Treated follicle showing damaged nuclear membrane. (H.E) ×960

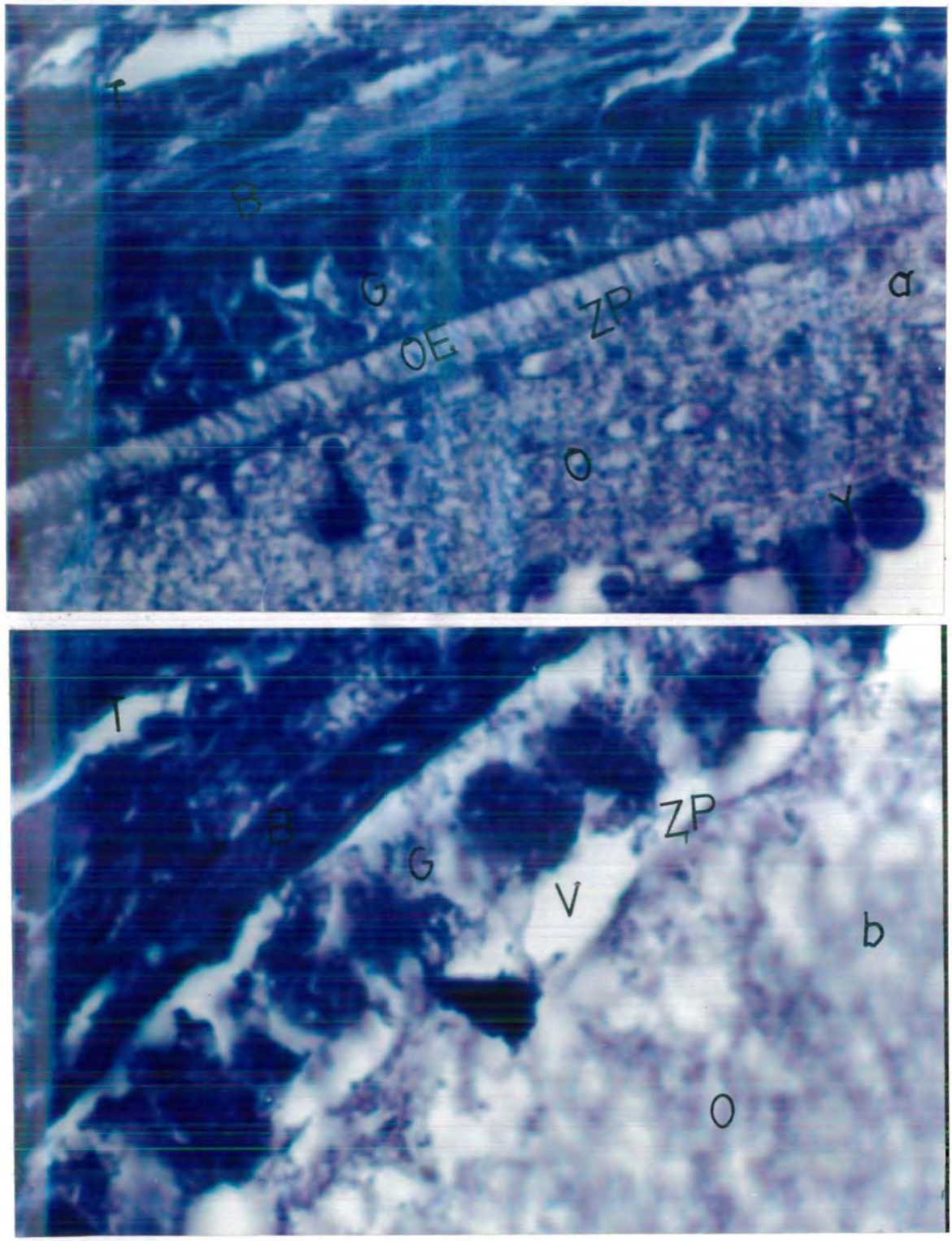
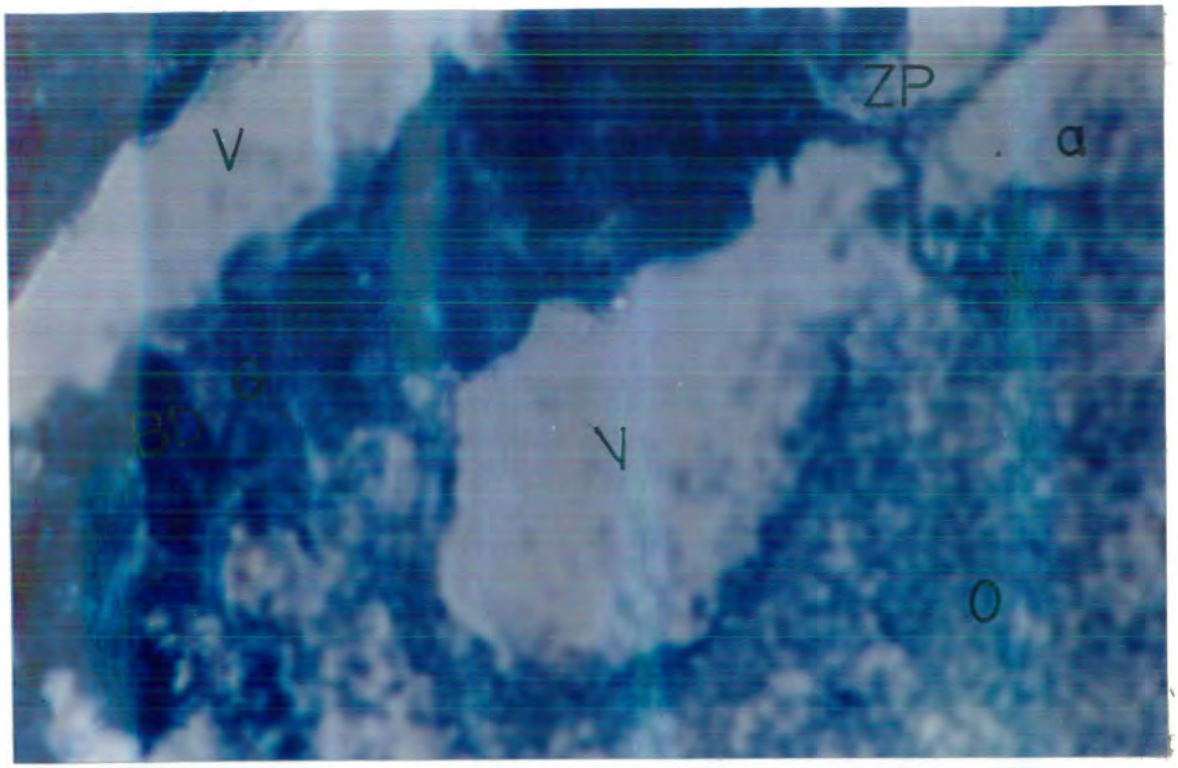


Figure 5: Photomicrographs of the middle portion of ovary of the Rhode Island Red bird at 24 weeks of age. a) Control ovary follicle showing theca layer (T) basal lamina (B) granulosa (G) ooplasma extension (OE) zona pellucida (ZP) ooplasm (O) yolk granules and. b) Showing follicles of treated birds thecal. b) Follicles of treated showing theca layer (T) basal lamina (B) granulosa (G) vacuolated region (V) degenerative zona pellucida (ZP). (H.E) ×2400



5c) Follicles of treated showing theca layer (T) vacuolated region between granulosa and theca (V) degenerative zona pellucida (ZP) darkly stained bodies (BD). (H.E) ×2400

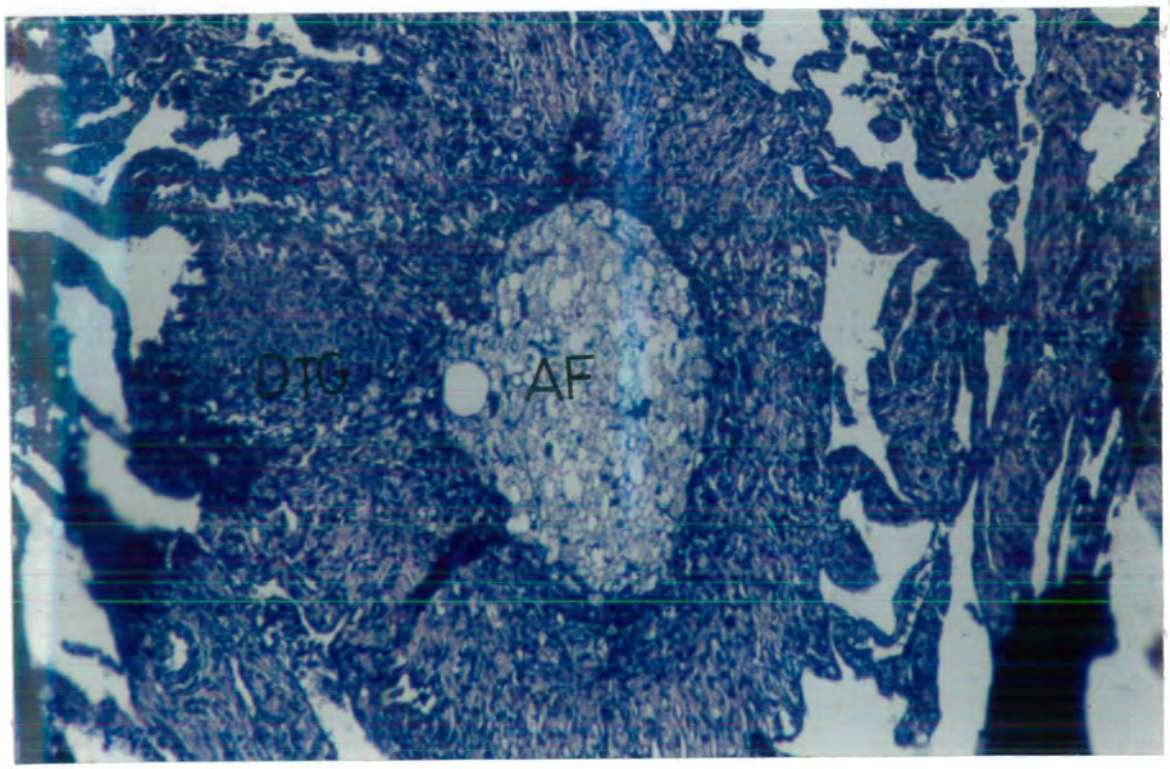


Figure 6: Photomicrographs of the middle portion of ovary of the Rhode Island Red furazolidone-treated birds at 24 weeks of age showing atrophic follicle with yolk atretic and no demarcation between thecal layer granulosa. (H.E) ×240

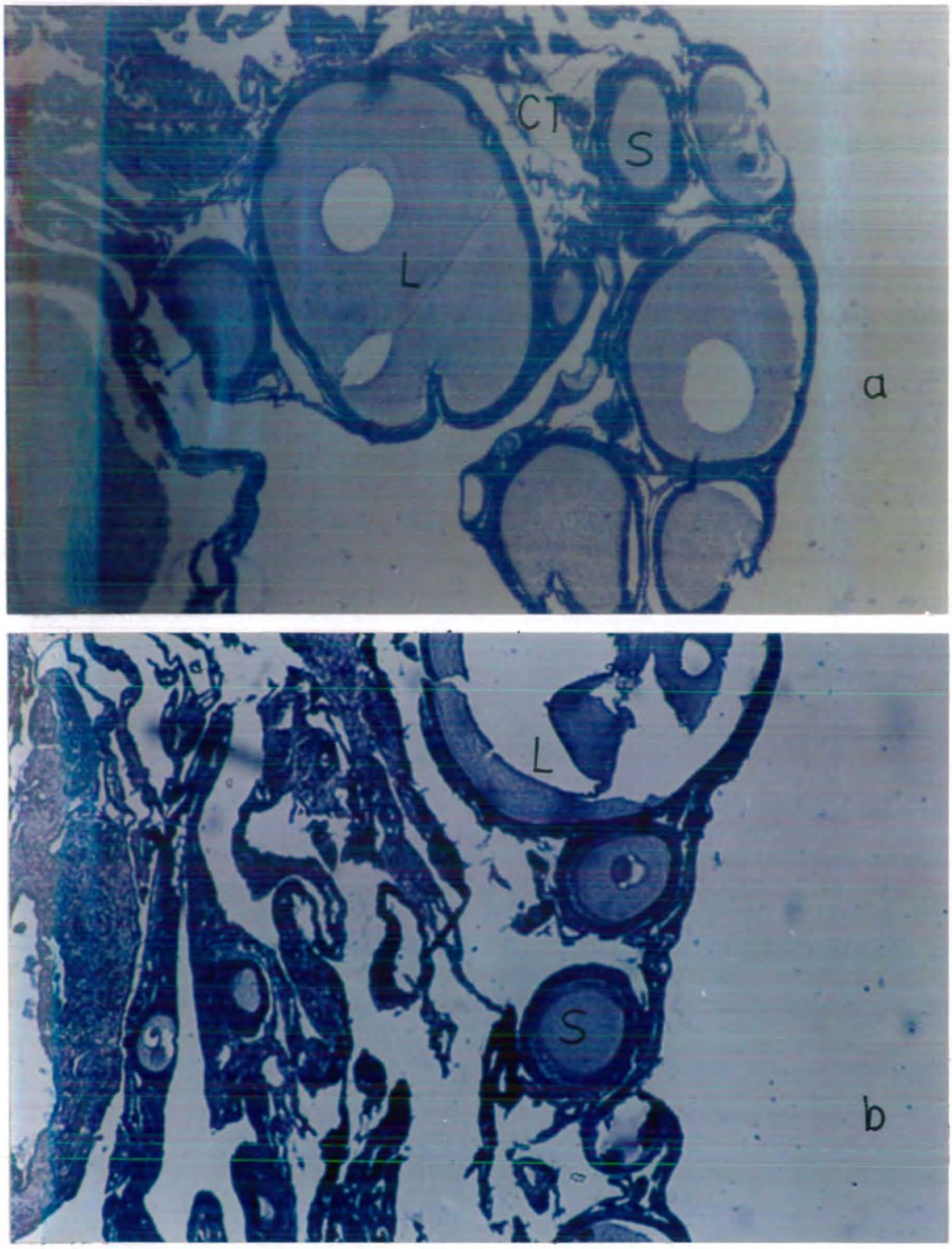
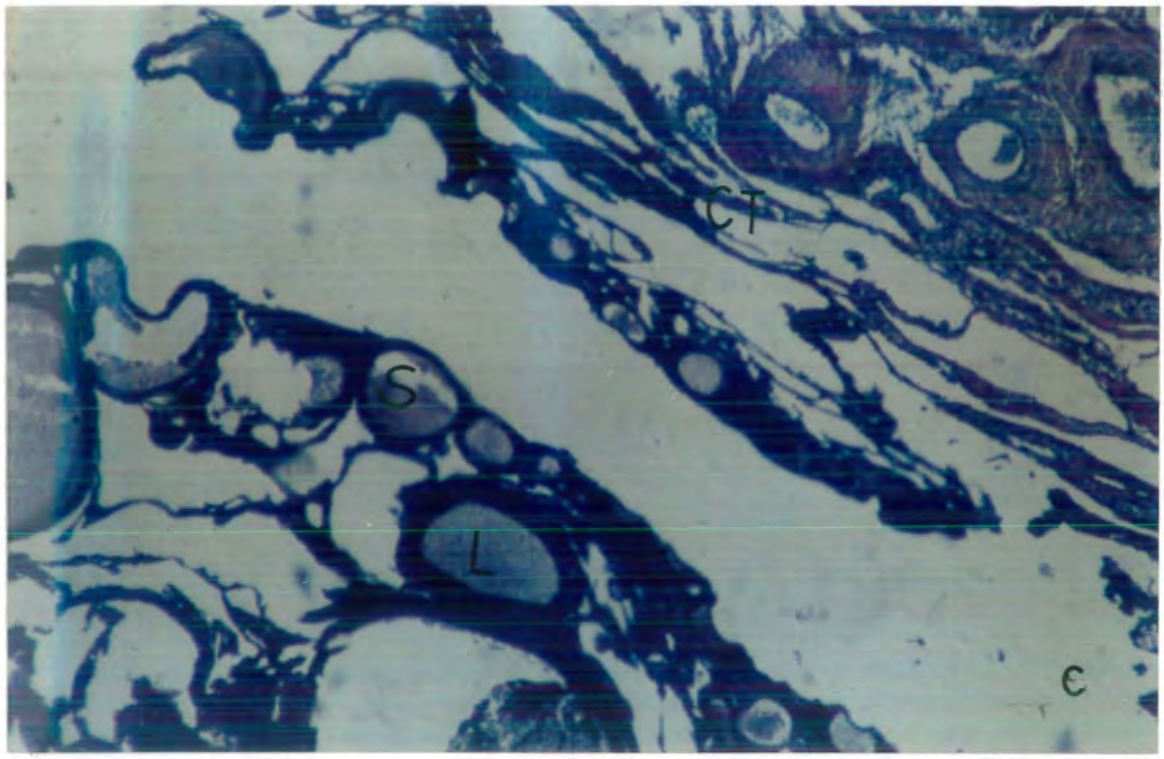


Figure 7: Photomicrographs of the middle portion of ovary of the Rhode Island Red furazolidone-treated birds at 24 weeks of age. a) Control ovary showing less small size (S)and larger (L) follicles on cortical region and connective tissue (CT). b) Furazolidone (400mg/kg feed) treated ovaries showing smaller (S) follicles on periphery region and loosely arranged connective tissue (CT) (H.E) ×240



b) Figure 7: Photomicrographs of the middle portion of ovary of the Rhode Island Red furazolidone-treated birds at 24 weeks of age. c) Furazolidone (800mg/kg feed) treated ovaries showing smaller (S) follicles on periphery region and loosely arranged connective tissue (CT). (H.E) ×240

CHAPTER

4

DISCUSSION

DISCUSSION

Nitrofuran is a class of drugs that has the ability to kill micro-organisms. The group consists of three drugs, namely nitrofuratoin, nitrofurazone and furazolidone (Brander et al., 1991). Furazolidone is a nitrofuran that has been used for more than forty years in the treatment of certain bacterial and protozoal infections in man and animals (Ali, 1999). In the present study effects of chronic oral administration of several doses furazolidone initiated during the pre-laying period of female Rhode Island Red birds, were investigated. The current study shows that chronic administration of furazolidone disrupted the pituitary-gonadal hormonal axis which changes the plasma concentrations of some reproductive hormones and also brought about the histomorphological changes and reduced egg production.

In present study birds were observed efficiently to notice the clinical signs of the furazolidone intoxication. No clinical signs of furazolidone toxicosis were examined. However, clinical signs and pathology of furazolidone toxicosis has been documented in chickens (Jesen *et al.*,1975; Salyi *et al.*, 1986). It is suggested that Rhode Island Red birds are more tolerant to furazolidone up to 800mg/kg than other avian species.

The effect of furazolidone-administration on body growth is reflected by variation in body weight of control and treated groups during the 5 weeks of treatment (15-20 weeks of age). A linear increase in body weight in all groups was observed when age of the birds was taken under consideration. The mean body weight in control and all treated groups were similar in first week of furazolidone-treatment. In second week of furazolidone administration depression in body weight gain was observed in group fed 800 mg furazolidone/kg. During 3rd, 4th and 5th week of administration, the body weight of groups fed 400mg furazolidone/kg feed and 800mg furazolidone/kg feed

were significantly ($P<0.05$; $P<0.01$ and $P<0.02$; $P<0.01$) lowered than the control birds. At the dose level 200 mg furazolidone/kg feed had no affect on body weight. Reduction in body weight gain was also recorded even after the withdrawal of furazolidone treatment, the gain in body weight was significantly ($P<0.01$; $P<0.02$) lower in group II during first and second week of furazolidone cessation. The reduction in body weight of group III during this period was also significant ($P<0.001$; $P<0.02$). Cessation of furazolidone feeding did not restore the body weight gain in group fed 800 mg/kg feed even after third week of furazolidone-administration withdrawn. The body weights of all groups were similar in forth week of furazolidone withdrawal. The present study suggest that decrease in body weight in birds fed different doses of furazolidone is dose and time dependent and also has reversible trend. This finding is similar to previous observation in different avian species Japanese quails, (Arbid *et al.*, 1990; Ullah *et al.*, 1998; Noorani *et al.*, 2001),chicks (Bartlet *et al.*, 1990; Andrabi *et al.*, 1998) and turkeys (Pang *et al.*, 1982; Czarnecki, 1989). It was also found in some other studies that furazolidone treatment suppressed body weight (Haq *et al.*, 1987; Vahls Strappers, 1985), they reported that furazolidone as a feed additive does not improve the body weight gain in broiler and care should be taken in recommending treatment with furazolidone at the end of the fattening period of birds.

A positive trend in gain in body weight was observed after the cessation of furazolidone -administration. However, gain in body weight was slow in the birds given high doses of furazolidone (400 mg and 800 mg). Body weight was comparable four weeks after furazolidone withdrawal. This finding correlates with the Noorani *et al.* (2001), they reported similar pattern of change in body weight gain in Japanese

quails after feeding furazolidone at the dose rate of 400mg and 600mg per kg feed for four weeks and basal feeding of birds for another four week.

Pattern of change in feed conversion ratio (FCR) in control group characterized by age related steady decrease, indicating that a better conversion of feed in body growth .However decrease in FCR was slow, showing that more feed is required by birds during pre-laying period. Where as in furazolidone-treated group a steady increase in FCR was observed, indicating poor conversion of feed in to body growth. FCR was significantly ($P<0.05$) high during 2^{nd} and 3^{rd} week of furazolidone in group III compared to control, showing decrease in conversion of feed in to body weight. In fourth and fifth week FCR improved in group I and but significantly ($P<0.02$) as compared to control. These findings differ from previous observations (Buksh et al., 1980; Haq et al.,1987), they reported furazolidone treatment was found to have positive effect on FCR in broiler chicks. This difference in observations might be due to different avian species was used in present study. However, the toxic effects of furazolidone on FCR has been reported (Czarnecki, 1989). In present experiment calculated feed was given to the birds once a day while in other studies the birds had the access to furazolidone-medicated feed ad libitum which could result in anorexia in birds.

Decrease in serum cholesterol concentration was recorded in this study. During Ist, 2^{nd} and 3^{rd} week of furazolidone treatment significant ($P<0.02$; $P<0.00$ 1) decrease in serum cholesterol concentration of group II and ($P<0.02$; $P<0.01$; $P<0.001$) of group III was observed. While in 4^{th} and 5^{th} week serum cholesterol level of group I fed 200mg/kg feed was also decrease significantly ($P<0.05$) as compare to control. The serum cholesterol level remained lower ($P<0.001$) in group II and III during 4^{th} and 5^{th}

week of furazolidone-treatment. The maximum decrease in serum cholesterol level was observed in group III at the end of fifth week of furazolidone treatment. Munawra (1998), reported the decrease in serum cholesterol level in young fayoumi chicks by giving (400mg/kg and 800mg/kg) furazolidone for three weeks at sixth, seventh and tenth week of age.

During the withdrawal period of furazolidone-treatment, steady rise in serum cholesterol level was observed in treated groups but serum cholesterol levels remained lower compare to control birds. The serum cholesterol level was remain significantly ($P<0.05$; $P<0.001$) lower in group I, II and III during Ist, 2^{nd} and 3^{rd} week of furazolidone withdrawal. Similarly, in 3^{rd} week of furazolidone cessation a significant ($P<0.05$; $P<0.01$; $P<0.02$) decline in serum cholesterol level of group I, II and III was observed. In 4th week of cessation of treatment, serum cholesterol concentration in group I and II was similar to the control birds. Serum cholesterol level in group III was significantly ($P<0.02$) lower compared to control birds. It has been found that both acetate and cholesterol may serve as precursors for the steroidogenesis in female and male birds (Turner and Bagnara, 1976). Ali *et al.* (1984a) observed the similar non-significant reduction in testicular concentration of cholesterol after giving dose of 40 and 80mg furazolidone per kg for five days. In the present study severe reduction in serum cholesterol level might be attributed to high dose of furazolidone and long duration of treatment. Decrease in cholesterol and aspartate aminotransferase (AST) activities (Oyejide *et al.*, 1983; Ali and Bartlet, 1982b) have also been described. It was known that 5-hydroxytryptamine (5-HT) in testes produce deleterious effects on this organ (Ellis, 1972). Ali and Bartlet. (1982a) also observed that furazolidone treatment significantly increased the 5-HT

concentration in chicken brain. This rise in 5-HT inhibits the monoamine oxidase (MAO) activity.

It has been established that thecal and granulosa cells of the developing follicles present all the indication of steroid-secreting cells (Lofts and Murton, 1973; Guraya, 1976). In general like that of the mammal the avian ovary produces steroid hormones (Gilbert, 1971; Loft and Murton, 1973; Murton and Westwood, 1977) . Estrogen was considered to be most probably produced by the thecal interstitial cell (Huang and Halbondov, 1978) but Chieffi and Botte (1965) argued that estrogens were produced by the granulosa cell and these are the main source of circulating estrogen and progesterone. The growing follicle secrete estradiol during the vitellogenic phase (Shodono *et al.*, 1975; Graber and Nalbandov, 1976) and progesterone shortly before the ovulation (Furr *et al.*, 1973; Etches and Cunningham, 1977). Estrogen also induces differentiation of the cells which synthesize egg white proteins e.g. ovalbumin and lysozyme. There are increase levels of estrogen during yolk deposition, preceded by increased levels of testosterone (Lance and Callard, 1978).

In the present study serum estradiol concentration was similar in all groups at the end of 1st week of furazolidone treatment. In 3rd and 5th week of furazolidone treatment serum estradiol concentration was significantly ($P<0.05$; $P<0.001$)affected in group II and ($P<0.02$; $P<0.001$) in group III. Serum estradiol concentration level showed little rise in these groups in 2nd week of furazolidone-treatment was withdrawn but significantly ($P<0.01$; $P<0.001$) lower than control birds. The serum estradiol concentration in last week of experiment approached to that of control birds. It is interesting to note that estradiol secretion in all group was observed to increase with age. Botte *et al.* (1966) incubated the thecal cell from growing follicles of the hen's

ovary and noted that these cell had the ability to convert cholesterol into estrogen. He further noticed that, there also increased level of estrogen during yolk deposition, preceded by increased level of testosterone and a surge of progesterone shortly before ovulation.

How furazolidone (some other nitrofurans) interfere with steroid production especially estrogens it is still matter of investigation because there is no report about the effect of furazolidone upon fowl estrogen levels available in the accessible literature. Although it is believed that the effects of furazolidone are largely attributable to the adrenal medulla (Ali, 1999). Jager *et al.* (1997) investigated that nitrofurans do have the ability to interfere with steroid hormone regulation and this was exemplified by aldosterone release from porcine and bovine adrenocortical cell *in vitro*. Senoir (1974a) found that in commercial layers estradiol concentration were maximum 6hr prior to ovulation during laying. The present results reveal that during the first two week of furazolidone-treatment estradiol levels were lower where as maximum estradiol concentration was observed in 3-5 week of experiment which is approximate laying time in these birds. The higher levels before laying are probably related to rapid formation and growth of ova (Senoir, 1974b). In some earlier study it was found that peripheral plasma show peaks at 18–22hr and 2–6hr prior to ovulation in laying hen estradiol concentration (Peterson and Common, 1972). In current study sampling was always done between 5:00–7:00 P.M to avoid the stress to the birds during day time which can effect the hormonal levels on weekly basis (Suttan et al., 1973; Pollard et al.,1980). The time of sampling indicates the mid of ovulatory cycle because under natural conditions of illumination, ovulation usually occurs in the morning and seldom takes place after 3 P.M (Turner and Bagnara, 1976). Senior

(1974a) observed in normal commercial layers that once the laying was established plasma estradiol concentration remained lower as compared to those prevailing before laying. Similar investigation was also reported by Hassan (1990) in Desi layers (*Gallus domesticus*) birds. In some further study it was found that nitrofurans and furazolidone increase plasma concentration of corticosterone in chickens (Ali, 1983; Bartlet *et al.*, 1990) and prolactin in turkeys (Ali *et al.*, 1987). Both hormones are known to cause stress in many species (Bassett *et al.*, 1973; El Halawani *et al.*, 1985; Ali, 1989). So it can be suggested that furazolidone adversely affect reproduction by decreasing estrogen secretion as observed in the treated group in the present study due to above mentioned stressor factors. Cessation of furazolidone feeding is known to cause restoration of spermatogenesis and normal function of the testes in chickens (Siddique *et al.*, 1996) and rise in the decreased levels of leutropin and prolactin (Ali *et al.*, 1987). Similar pattern of changes in the estradiol peripheral plasma concentration was observed in present experiment after the withdrawal furazolidone feeding. It is established that LH is responsible for estrogen secretion by stimulating thecal cell of the ovary however, the exact mechanism that how ovarian steroidogenesis is regulated by FSH and LH in birds is not well understood (Lance and Callard, 1978).

Furazolidone-administration at dose level 400mg and 800mg/kg feed for 5 weeks significantly affected serum LH levels. Serum LH concentration was similar during first week of treatment. Furazolidone-treatment significantly ($P<0.05$) decrease serum LH concentration of group III in 3rd week. During fifth week the serum LH furazolidone showed significant ($P<0.05$) decrease in group II and higher concentration of furazolidone showed significant ($P<0.014$) decrease in group III. A

significant ($P<0.05$) decrease in serum LH of group II and III was also observed during 2nd week after the withdrawal of furazolidone. In 4th week of furazolidone withdrawal serum LH concentration remained significant ($P<0.001$) lower in all treated compared to control birds. Similar, finding was obtained by Ali *et al.* (1987) after administration of furazolidone at a concentration of 15mg/kg for seven days in female turkeys. They also reported that furazolidone-treatment significantly reduce serum LH concentration which was remained lower seven days after withdrawal of the furazolidone. LH peak was detected in the plasma of laying hen 4-6hr before ovulation (Furr *et al.*, 1973; Etches and Cunningham, 1977). Nelson *et al.* (1965) reported three LH peaks in plasma. Two occur 13 and 8hr prior to ovulation and these are essential for ovulation. First of three peaks occurs 21hr prior to ovulation. The decrease in LH concentration in present study would be expected to decrease in the secretion of ovarian steroids and reproductive activity. The drop in egg production was observed during the treatment period probably the outcome of decrease in LH serum levels. Furazolidone and nitrofurazone also increases the prolactin concentration in plasma of birds (Ali *et al.*, 1987). This property of the drug considered as the effect on adenohypophysis or could be a consequence of the stressor action of the drugs. The furazolidone has been termed as a "stressor" agent in chickens because of its action on adrenal gland and plasma corticosterone (Sidorov and Luders, 1971). The decrease in LH could have resulted from a direct effect on the adenohypophysis. However, this effect also be related to altered hypothalamic amines which are known to involve in the reproductive hormones and alleviate the concentration of catecholamines in the turkeys hypothalamus (El Halawani *et al.*, 1982).

Furazolidone-administration delays egg laying in the birds on dose dependent manner. In present study birds were given different doses of furazolidone for five weeks which significantly ($P<0.003$) decreased the egg production in treated groups. The egg production significantly ($P<0.001$) increased with the advancement in age of the birds. In control group egg laying was initiated in eighteenth weeks of age which is optimum age of egg laying in number of avian species (Gilbert, 1971) but in the treatment group I and II the laying of eggs was delayed and egg production started in 19th and 20th weeks of age in group II and III depending upon the dose of furazolidone. This is in agreement with the results obtained by Babu *et al.* (1994) who reported that furazolidone even at low level 50 ppm and 100ppm delays in attaining the sexual maturity of white leg horn. Egg production is affected variably in other species given furazolidone. These investigations are similar to the Ali *et al.*(1987) who found that furazolidone at dose level 15 or 30 mg/kg markedly reduced the egg production in turkeys. In some other study it was also investigated that, diet containing more that 400mg/kg furazolidone severely reduced the egg production in Japanese quails (Dixon *et al.*, 1992). These findings are contrary to others (Cooper, 1956; Franis and Shaffner, 1956; Belloff *et al.*, 1958; Kondra and Guenter, 1968; Ehlhardt *et al.*, 1975) who described that furazolidone up to dose level 500mg/kg did not effect the egg production in domestic fowl. This difference could be due to longer duration of furazolidone-administration and initiated during pre-laying age of birds in present study. The furazolidone in the diet is known to have an effect on food palatability and intake (Belloff *et al.*,1958; Ali *et al.*, 1984) and reduced food intake results in drop in egg production (Arzey, 1987). Furthermore, reduction in serum LH concentration caused directly by furazolidone-administration would decrease the

secretion of ovarian steroid, which might be one of the cause of reduce egg production. Babu *et al.* (1994) used furazolidone low level (100ppm) furazolidone use as supplementation in layer ration. This supplementation of furazolidone for 40 weeks resulted in extra egg production than control birds. In present investigation the 200mg furazolidone/kg feed which is near to the above level did not effect the egg production as it was given for five weeks only.

Present study also shows that furazolidone treatment did not affect the egg weight, volume and size. A significant ($P<0.0001$) increase in mean egg weight volume and size was observed with advancement of age but synergistic effect of furazolidone-treatment and age of the birds was observed on mean egg weight and volume. There was no abnormality in shape and texture of egg shell. This might be the reason for the synergistic effect of furazolidone-treatment and age of the birds. There are also some reports of reduction in egg size in domestic fowl fed furazolidone at level up to 400mg/kg (Sauer *et al.*, 1969; Ehlhardt *et al.*,1975). The size of egg increased with the advancement of age of birds which is natural phenomena in laying hens. In present study furazolidone-administration was discontinued after five weeks and a period of four weeks was given for withdrawal of drug. So it can be said that during withdrawal period, furazolidone level in the body of the birds is insufficient to effect the egg size, weight and volume. Botsglou *et al.* (1989) fed 100ppm, 200ppm and 400ppm furazolidone for different duration. They observed the deposition of furazolidone in the egg up to 11 days in the birds fed high dose of furazolidone.

In current study the birds were given furazolidone for five weeks between the age of 15-20 weeks and after withdrawal period of four weeks birds were sacrificed at the age of 24 weeks. Furazolidone-treatment did not effect the total ovarian weight (small

and large follicles) at any dose level in present study. Similar changes in ovarian weight have also been recorded by Arbid *et al.* (1990) in ducks and Ullah *et al.* (1998) in Japanese quail. These changes were reversible after cessation of furazolidone feeding and became non-significantly different from control group. Where as furazolidone caused significant ($P<0.01$) decrease in ovarian weight with small follicle, and ($P<0.01$; $P<0.02$) volume in groups fed high doses indicating non reversible change. The size of the ovaries was observed similar to the control group. Such results have so for not been reported by any other scientist. Adverse effects of furazolidone-treatment on testes weight have been reported in male domestic fowl (Quesada *et al.*, 1986; Andrabi *et al.*, 1998). Arid *et al.* (1990) found that ovaries become severely atrophied after feeding furazolidone 600ppm and 800ppm for two weeks and became misshapen as in immature birds.

It was observed that oviduct length reduced significantly ($P<0.05$; $P<0.01$) in group II and III given 400mg and 800mg furazolidone respectively, at the end of experiment while oviduct weight remained unaffected. Oviductal length and diameter were markedly reduced in the birds treated with 800ppm furazolidone for two weeks in Japanese quails (Arbid et al., 1990; Ullah et al., 1998). No reports quoted in the accessible literature about pathomorphological changes induced by furazolidone in the oviduct of fowl. However, the functional development of the avian oviduct requires multiple steroid hormones. The full development of magnum, secretes the albumin around the ovum produced by estrogen (Turner and Bagnara, 1976). The exact mechanism by which furazolidone induce these morphometeric change in oviduct are not known. Palmiter and Wrenn (1971) investigated that estrogen triggers the cytodifferentiation of tubular gland cells in the magnum portion of the oviduct

epithelium. Similar finding are also obtained by Kohler *et al.* (1969) that estrogen induce cytodifferentiation in the ovalbumin glands in the chick oviduct. During the magnum growth, plasma estradiol and progesterone concentration followed the different patterns in quail in the process of sexual maturation i.e initiation of epithelium cell proliferation occurs in sharp decrease in plasma progesterone and increased estrogen levels at the same time (Pageaux *et al.*, 1984). Any abnormality in estrogen and progesterone hormonal concentration caused involution, decrease in total weight and abnormal oviduct functions (Oka and Schimke, 1969). The decrease in length of oviduct in treated birds might be due to decrease peripheral levels of steroid hormone which was observed in present study. Taken to gather the results and present study suggest that furazolidone treatment exerts significant adverse effect on gonadal hormones but probably insufficient to significantly alter the oviduct weight and volume in chickens. It is also possible that withdrawal period of four week are sufficient to induce reversible effects in poultry birds.

It is also established that combs and wattles length, width and weight were not significantly ($P<0.05$) affected by the furazolidone treatment. Ali *et al.* (1984a) obtained similar results in male chickens at the dose level 0.04% w/w for ten days but at the dose level 0.08% w/w for the same duration significantly reduced the weights of combs and wattle. In present study withdrawal time of four week was given to birds and then data was collected. Furazolidone has reversible effects due to this property of drug suggesting that reduction in size of combs and wattle is also reversible and was approaching to control group. The development of the secondary characters are regulated by the continues secretion of androgen (Turner and Bagnara, 1976; Parkhrst and Mountney, 1988) and indicate of age of sexual maturity (Karpp,

1918). Furazolidone decreased androgen secretion in male birds (Ali *et al.*, 1984; Andrabi *et al.*,1998). A non-significant decrease in length, width and weight of wattles and combs in furazolidone-treated group attributed to suppressed estrogen secretion in these groups. These results suggest that furazolidone-administration at dose level (200mg, 400mg and 800mg furazolidone kg/feed) for 5 weeks during pre-laying period exerts significant adverse effects on endocrine and exocrine portion of ovary but probably insufficient to markedly alter the growth of secondary sexual characters of birds. Earlier, Cooper and Skulski (1956) investigated that furazolidone-administration at dose 004% w/w for 14 days to immature male chickens resulted in a significant reduction in the weights of the combs.

Friable consistency of liver in groups fed high doses (400mg and 800mg) was noted but the weight of liver remained non-significantly different among all groups. This difference in consistency of liver in different groups could be attributed to monoamine oxidase (MAO) inhibitory activity of furazolidone in the tissues of chickens, turkeys and ducks (Ali,1989). Contrary, in quails furazolidone toxicosis is reported to decrease liver body mass significantly (Arbid *et al.*, 1990) In some earlier study Orr *et al.* (1986) found that furazolidone causes vacuolation of cytoplasma of hepatocytes and biliary ductular hyperplasia. These difference in observation might be due to species variation and withdrawal period of four weeks was given in present experiment.

The present study reveals that furazolidone significantly affected the oocyte development. The mean number of the oocytes in the range of diameter 101-200μm was significantly (<0.05; $P<0.02$; $P<0.001$) lower in all treated groups. The proliferation of oocyte in the range of 201-400μm were significantly ($P<0.05$; $P<0.01$;

P<0.001) reduced in groups treated depending upon the dose of furazolidone. It was also observed that mean oocyte number in the range of 401-500μm diameter were significantly (P<0.01) lesser in group III. The mean number of oocytes in range of 501-600μm were significantly (P<0.02; P<0.01) lower in group II and III. It was interesting to note that follicles of the diameter in the range of 601-700μm were not seen in the group II and III given higher dose of furazolidone but found in group I fed lower dose of furazolidone. Mean number of follicles in the having diameter in the range of 701-800μm were on seen in all treated all groups. The ovary of the laying hen contains several million follicles (Hutt, 1949) of which several thousand are microscopically visible (Pearl and Schoppe, 1921). The present study is concerned with both microscopic and macroscopic follicles. These results reveal that furazolidone treatment effects markedly the follicles in the range upto 201-400μm. Similar finding was reported by Munawra (1998) that furazolidone at 400mg/kg and 800mg/kg significantly reduced the mean follicles number in the range of 301-400μm and 401-500μm in young Fayoumi birds. The decrease in mean number of oocyte in higher range in treated groups could be due to lesser development of follicle at early stage or it could be that there is tendency for the number of follicles in each size class to decrease with increase in size of the follicle (Gilbert et al., 1983). This data reveals that furazolidone-treatment effects the follicle growth in the range above 100μm in female birds.

Furazolidone-administration caused significant (P<0.05; P<0.001) reduction in follicle number having granulosa layer thickness in the range of 1-10μm group I, II and III. In furazolidone treated birds follicular granulosa layer the thickness in various

categories ranging from 11-50μm showed no significant difference in follicular number compared to from control birds. No information is available in accessible literature regarding effect of furazolidone on thickness of granulosa layer. However, according to Munawra (1998) furazolidone treatment at the dose level 400/mg and 800mg/kg feed resulted non-significant change in granulosa layer thickness of Fayoumi chicks upto the age eight weeks but significantly increases the granulosa layer thickness in birds at the age of ten weeks.

The mean nuclear diameter of the oocyte was significantly affected by furazolidone-administration at higher doses. The mean number of oocytes having nuclear diameter in the range of 21-40μm were significantly ($P<0.05$; $P<0.01$) decreased in group II and III compared to control. There was also significant ($P<0.01$; $P<0.001$) decrease in the mean number of oocyte having the nuclei size in the range of 41-60μm in diameter. The oocyte with 60-80μm nuclear diameter were seen only in control birds. Whose oocytes were also greater (601-700μm and 701-800μm). This probably might be the one of the reason for the presence of greater size (60-80μm)of follicular nuclei in control group only. Above mentioned effects of the furazolidone on the oocyte nuclei are describe for the first time.

The present study reveal that mean number of yolky follicles decreased with increase in the size of follicles. The mean number of yolky follicles in the range of 1-5mm and 6-10mm were significantly ($P<0.01$; $P<0.05$) lowered in all treated groups. Similar in all group. The mean number of yolky follicles in the range of 6-10mm were also significantly ($P<0.01$; $P<0.05$) lower in group I, II and III. The higher concentration of furazolidone significantly ($P<0.001$; $P<0.05$) reduced the mean number of yolky follicle in the range of 21-25mm in group III compared to control birds. There was no

difference in mean number of follicle in the range of 26-30mm among all groups. Gilbert et al.(1983) and Perry et al. (1983) reported that follicular maturation takes within fourteen days during which time follicles increases in diameter from about 1-40mm and ovulate. It was found that at a time more than ten follicles of 2-3 mm diameter may be present but at most 1 or 2of these are certain to ovulate and follicle up to 6mm or so (small yolky follicles) susceptible to atresia, follicle larger than about 8mm (large Yolky follicles normally grow to ovulate (Fell, 1923). This study suggests that furazolidone interfere with development of the follicle in the range of 1-10mm diameter. This might be possible reason for decrease number of follicles in the of 21-25mm in treated RIR birds.

The ovary of the control birds was significantly ($P<0.01$) large in size due to presence of large yolk follicles. The diameter of the follicles in the range of 1-30mm. Similar proliferation of follicles in treated groups were also seen however, their number was lower. The large follicles decrease in number with increase in size of follicle. According to (Gilbert et al., 1983) that follicles have the tendency to decrease in number in each size with increasing size of the follicle. These large follicles attached to surface of the ovary with the help of distinct stalks. In addition to these yolky follicle small white follicles in the range of (1-5mm) were also observed on the ovarian surface of both control and treated birds. Waddington and Walker (1988) observed of the distribution of different size of the follicles in various region in ISA Brown strain ovary at different ages. They found that posterior segment of the ovary of birds of 30, 45, and 86 weeks of age contained more follicles in rapid growth phase in the range of 1-4-8mm diameter. Although it was found that FSH is almost certainly involved in the follicular growth (Gilbert, 1971). However its precise mechanism and

its site of action within the ovary are unknown.

It was also observed that furazolidone induce various changes in the organization of the ovary in follicles in the range of 1-600μm diameter. In control birds larger follicles were present on cortical region where as in treated groups smaller follicles were present on cortical region. Stromal tissue and connective tissue were loosely arranged in treated birds. Disrupted zona pellucida and vacuolated region between zona pellucida and granulosa and no cytoplasmic extension in treated group was seen. The nuclear membrane was also damaged in treated birds. The effects of furazolidone on chick ovarian histomorphology and toxicosis has not been in adult bird in literature. However, it was found that furazolidone induced similar alteration in the organization of ovary in young (6^{th}, 8^{th} and 10^{th} week of age) female Fayoumi chicks (Munawra, 1998). Some lightly and darkly stained cells were seen in the granulosa layer of follicles epithelium of the control and treated birds. Guraya (1976) and Ballairs (1965) demonstrated these darkly stained follicle cells in various avian species and denoted them the degenerating cells as they are gradually resorbed.

The present data provides the information regarding several aspects of furazolidone toxicosis in layer birds which hitherto have received little attention. Therefore, this data warrant poultry enterprises that care should be taken in usage of furazolidone-administration regarding its dose and duration at pre-laying period of the birds.

CHAPTER

5

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