PHYSIOLOGICAL EVALUATION OF DIETARY SUPPLEMENTATION OF STEROID HORMONES AND ALPHA-TOCOPHEROL IN BROILER CHICKEN

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DECLARATION

I here by declare that the thesis entitled **"PHYSIOLOGICAL EVALUATION OF DIETARY SUPPLEMENTATION OF STEROID HORMONES AND ALPHA-TOCOPHEROL IN BROILER CHICKEN"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"PHYSIOLOGICAL EVALUATION OF DIETARY SUPPLEMENTATION OF STEROID HORMONES AND ALPHA-TOCOPHEROL IN BROILER CHICKEN"** is a record of research work done independently by **Dr. Cyel .V. Yohannan** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

The animal production can be improved by increasing the growth rate and feed conversion ability. Growth rate of animals and birds can be improved by the administration of several dietary agents such as micronutrients (vitamins), antimicrobial compounds (probiotics and antibiotics) and certain hormonal preparations of either natural or synthetic origin.

Both the endogenous and exogenous hormones can influence the performance of animals and birds. Endogenous steroid hormones like estradiol, progesterone, and testosterone are produced in the gonads. The naturally produced endogenous steroids are not orally active, require relatively large doses to be administered to get the physiologic response, and can transiently affect the behavior of treated animals. In addition to natural steroids, synthetic steroids are also used because they are generally more potent, have less androgenicity, and thus have less adverse effects on behaviour. The synthetic steroids used are the preparations of either androgenic as trenbolone acetate (TBA) or progestogenic (Melengestrol acetate) or nonsteroidal estrogens (stilbene estrogens and phytoestrogens).

There are number of reports in the media regarding the use of different growth promoters including anabolic steroids (hormones) by poultry farmers in broiler chicken. Reports on the side effects of administration of various estrogenic preparations in human beings and possible impact of their residues in meat animals resulted in public outcry regarding the safety of these compounds and prompted regulatory agencies like European Union to ban the use of hormones as growth promoters (Directive 88/146/EEC). Scientific information regarding the influence of anabolic steroid supplementation on the growth performance or other physiological/biochemical profile of broiler chicken is scanty.

Vitamin E is one of the four fat-soluble vitamins. This vitamin is synthesized by plants and stored in plant oils, and has eight different isoforms (vitamers) divided

into two classes of four vitamers each. These compounds are comprised of a 6-chromanol ring and an isoprenoid side chain. Compounds having saturated side chains are classified as tocols. The second class of compounds, known as tocotrienols (trienols) have unsaturated side chains. The groups attached to the R1, R2 and R3 positions on the 6-chromanol ring designate the vitamer as alpha, beta, gamma, or delta. A large body of the research currently focuses on the α-tocopherol form of vitamin E, which is the most biologically active form. Less expensive form of vitamin E designated as dl-α-tocopherol is commercially available.

The primary role of vitamin E within the body is to function as an antioxidant and considered as one of the major chain breaking antioxidant in membranes. The antioxidant property of vitamin E is coined to its membrane lipid stabilizing ability and there by reducing the impact of oxidation injury to cell wall lipid components. Systemic oxidation injury has been linked to numerous possible conditions/diseases including: cancer, aging, arthritis, atherosclerosis and cataracts. Alpha-tocopherol is having the antioxidant property can provide optimum performance in broiler chicken under various stress conditions.

Although much literature is available on the effects of supplementation of various oestrogenic preparations and vitamin E on the metabolism of humans and various animals, meager information is available on the effect of dietary supplementation of steroid hormones (oestrogen and progesterone) or vitamin E (α-tocopherol) or their combination in poultry. Hence, the present study was conducted with following objectives:

1. To study the effect of steroid hormones (Ethinylestradiol and Levanorgestrel) and α-tocopherol supplementation on growth in broiler chicken.

2. To assess the histomorphological changes in liver, adrenal glands and breast muscle.

3. To monitor the changes in haematological and biochemical parameters induced by steroid hormones (Ethinylestradiol and Levanorgestrel) and α-tocopherol.

Review of Literature

2. REVIEW OF LITRATURE

The efficiency of production in animals and birds can be improved by increasing growth rate and feed conversion ability. The growth rate of animals can be enhanced by feeding micronutrients such as vitamins and antimicrobial compounds that change the population of microorganisms in the gastrointestinal tract of healthy animals, resulting in improved animal performance.

During the last decade of the current century due to improvements of weight gain and feed efficiency in meat producing animals, administration of exogenous sex steroids has been a practice followed by farmers in many parts of the world. The most widely used substances are estrogens, either in the form of estradiol-17β, estradiol benzoate, or the syntheic zeranol. Progesterone, testosterone and the two synthetic hormones trenbolone acetate and melengestrol acetate are generally used in combination with estrogen (Anderson and Skakkebaek. 1999) in meat producing animals. The available literature with regard to use of ethinylestradiol in poultry is scanty and hence in most case the review uses data available with estrogen use in other species.

2.1 EFFECT OF EXOGENOUS ESTROGEN AND PROGESTERONE IN BROILER CHICKEN

2.1.1Effect on Growth

Estradiol-17β implants increased weight gain in steers while they did not increase weight gain in bulls. The improvement in weight gain was probably due to metabolic efficiency rather than an increased feed intake (Lee *et al.*, 1990). Average daily gain improved in response to estradiol and it also increased carcass weight and this was partially due to increased growth hormone secretion as well as somatomedin-C. Estradiol increased somatomedin-C by increasing growth hormone secretion in addition to increasing somatotropic receptors in liver (Enright *et al.*, 1990).

Rumsey *et al.* (1990) suggested that a measurable response to estrogenic implants could occur only over the dry matter intake range above maintenance intake of adequately balanced diets as estrogen is found to increase the BMR in beef steers. Rumsey *et al.* (1992) also opined that implants containing estradiol benzoate and progesterone improved body weight gain, empty body gain for water and protein as well as showed a trend towards greater protein and less fat deposition. According to Hutcheson *et al.* (1992) average daily gain, live weight (14%) and carcass weight (12%) were greater in Zeranol implanted lambs compared to controls. In the same experiment they also observed that Zeranol showed no influence on dressing percentage among lambs while it increased leg score.

Sadek *et al.* (1995) reported that poultry farmers in Egypt practiced mixing of ethinylestradiol pills in ration as a growth promoter. Preston *et al.* (1995) observed increase in average daily gain and gain efficiency when feed lot steers were implanted with combination of estradiol benzoate, progesterone and trenbolone acetate. Kniffen *et al.* (1999) observed no difference in body weight and carcass traits during growing as well as finishing phases of heifers implanted with estrogen. They also found that the relative growth rate tended to reduce both in single and double implanted heifers. Commercial implant Synovex S (Estradiol benzoate and Trenbolone acetate) increased dressing percentage, hot carcass weight, longissimus muscle area (Platter *et al.*, 2003).

Perinatal exposure of phytoestrogens (Whitten *et al.*, 1993; Casanova *et al.*, 1999) and ethinylestradiol are reported to reduce growth of rat offspring's as well as in exposed mothers (Masutomi *et al.*, 2004). Mader. (1994) reported that delaying estrogen administration till the animal has reached late growing period or using a low dose implant during an initial feeding period provides performance enhancement in subsequent feeding periods. He also noted that high dose implants of estrogen have more effect on weight gain during the growing phase than the low dose implants. In

the same experiment he found that trenbolone and estrogen has lesser effect on performance than when estrogen alone was used.

2.1.2Effect on feed consumption

Lee et al (1990) found out that Estradiol-17 *ß* implants didn't change feed intake in steers and bulls. Hunt *et al.* (1991) observed that estradiol implants in steers has no effect on the feed efficiency. Rumsey *et al.* (1992) observed a trend for increase in dry matter intake and feed conversion in steers treated with implants containing estradiol benzoate and progesterone. They found that the intake:gain ratio was also improved by the implants. Estradiol benzoate in combination with TBA improved feed efficiency in steers (Foutz *et al.*, 1997).

2.1.3 Plasma profile of estrogen.

Renema *et al.* (1999) found that broiler breeder stock showed variation in their plasma profile of estradiol-17 beta among birds kept in differing feeding level. The value varied from 40.7 and 38.1 pg/mL respectively for adlibitum fed group and restricted group at photosensitization (day 21) while at sexual maturity the values were 104.5 and 94.4 pg/mL respectively. There was no difference in the mean values (113.3 pg/mL). In the same study itself they found a relation between body size and estradiol-17beta level. The values were 35.5 and 49.1 pg/mL respectively for standard and high size birds and at sexual maturity it was 85.7 and 115.1 pg/mL respectively. The mean values were 108.3 and 134.1 pg/mL respectively for standard and high size birds. Serum concentrations of estradiol 17beta were increased in pigs with estradiol 17 beta implants (Rempel and Clapper. 2002).

2.1.4 Effect on lipid profile

According to Meade *et al.* (1977); Fotherby and Caldwell (1994) progestogens and estrogens in oral contraceptives have partially opposing effects on the metabolism of plasma lipoproteins in human beings. Ethinylestradiol stimulates the hepatic synthesis of triglycerides, apolipoprotein (apo) B and apo A1. Ethinylestradiol thus enhanced the production of both very low-density lipoproteins

(VLDL) and high-density lipoproteins (HDL). At the same time, ethinylestradiol increased the expression of low-density lipoprotein (LDL) receptors. However, enhanced production and enhanced catabolism of LDL together accelerated the turnover of LDL. The pronounced decrease in HDL_2 cholesterol observed in the ethinylestradiol and levanorgestrel group reflected the enhanced catabolism of HDL_2 stimulated by levanorgesrel in human beings (Tikkanen *et al.*, 1982, Kuusi *et al.*, 1985). Ethinylestradiol increased the concentration of HDL and, in particular of HDL2, also by suppressing hepatic lipase, which was responsible for the conversion of HDL2 into HDL3. Androgens and progestogens with androgenic properties, in contrast, increase hepatic lipase in human beings (Tikkanen and Nikkila., 1986; Krauss and Burkman., 1992). According to Notelovitz *et al.* (1989), Janaud *et al.* (1992) and Endrikat *et al.* (2002) oral contraceptives containing ethinylestradiol in combination with the progestogen levonorgestrel tend to raise LDL concentrations and to lower HDL and HDL2 in particular in women.

Schaefer *et al.* (1983) and Machado *et al.* (2004) observed that treatment with potent estrogens increases the plasma concentrations of triglyceride and the components of HDL and decreases those of LDL in women. Park and cho (1988) confirmed that estradiol implants in chicks resulted in marked elevation of all major plasma lipids with greatest increase in triglycerides (TG) followed by phospholipid (PL) and cholesterol. The results of Cho *et al.* (1988) confirmed that young male chickens implanted with estrogen for three week developed a marked hyperlipidemia. Plasma levels of triglyceride, cholesterol and phospholipid were elevated 68, four and 24 times, respectively, than the control chicken.

Cho and Park, (1990) compared estrogen treatment in fed birds as well as fasted birds with its control resulted in a marked elevation of plasma lipids, especially the triglyceride level during the two day period. Even in chicks fasted for five days; estrogen treatment resulted in a persistent hypertriglyceridemia. In fed chicks, estrogen treatment also induced a fatty liver with massive accumulation of

triglyceride, but the liver of estrogen treated-fasted chicks appeared to be normal. Williams *et al.* (1990), Haarbo *et al.* (1991), Bjarnason *et al.* (1997) and Adams *et al.* (1997) reported that oestrogen treatment was associated with a reduction in total plasma cholesterol in several animal studies. While few authors as Wagner *et al.* (1991) and 1997, Sulistiyani *et al.* (1995), Hanke *et al.* (1996) and Haines *et al,* (1999) differed in their views. Wagner *et al.* (1996) noted that esterified estrogen with and without methyl testosterone lowered total plasma cholesterol, LDL,VLDL and IDL in cynomegalous monkeys challenged with atherogenic diet. The treatment also reduced arterial LDL degradation and cholesterol ester content.

Campos *et al.* (1997) reported that the hypertriglyceridemia in postmenpausal women was associated with daily administration of oestrogen (2 mg estradiol 17ß) in the micronized form for a period of six weeks resulted in an increase in light, or buoyant, very low density lipoprotein (VLDL). Smaller, denser, and less buoyant forms of lipoproteins, including dense VLDL and intermediate density lipoproteins, did not increase in their concentrations. The reduction in LDL was associated primarily with the reduction in light LDL with no change in the smaller, denser LDL. Compared with controls, a significant increase was observed in the plasma levels of total triglycerides (24–78%), total phospholipids (7–20%), very low density lipoprotein (VLDL) triglycerides (61–76%), VLDL-phospholipids (14–60%), low density lipoprotein (LDL) triglycerides (8–35%), LDL-phospholipids (28–30%), high density lipoprotein (HDL) cholesterol (8–16%), HDL 3-cholesterol (11–20%), HDL-triglycerides (17–66%), HDL-phospholipids, HDL 3-phospholipids (7–11%), apolipoprotein (apo) A-I (5–20%) and apo A-II (10–40%) during treatment with formulation containing ethinyl estradiol and gestodene/norgestimate (Wiegratz *et al.*, 1998). According to de Valk-de Roo *et al.* (1999) conjugated equine estrogen showed no effect on total cholesterol while it reduced LDL and increased HDL (from six months) and TG (from twelve months) in postmenopausal women.

Walsh *et al.* (2000) observed a decrease in basal LDL accumulation rates and endothelial layer permeability in arteries from estradiol-treated animals. They suggested two independent mechanisms of anti-atherogenic protection by estradiol by decreasing endothelial layer permeability; and 2 incorporating estradiol into the LDL particle and there by preventing LDL binding to the artery wall.

Neither subcutaneous estradiol deconate nor oral 17-β estradiol affected the mean plasma cholesterol, VLDL or TG concentration in overiectamized rabbits (Zandberg *et al.,* 1998). According to Crook and Godsland (1998) and Scharnagal, *et al.* (2004) combination of ethinylestradiol with higher doses of levanorgestrel may cause an increase in LDL cholesterol and a decrease in HDL cholesterol. Treatment of rats with ethinylestradiol lead to marked increase in the number of LDL-receptors, resulting in lower plasma LDL-cholesterol levels as a result of increased clearance (Lansink *et al.,* 1999). Peverill *et al.* (2001) reported that oral combined hormone replacement therapy (HRT) with estradiol and norethisterone resulted in a 12% decrease in total cholesterol and a 11% decrease in LDL cholesterol.

Oral estrogen administration reduce the level of total and low density lipoprotein LDL cholesterol and increase high density lipoprotein (HDL) cholesterol and triglyceride. These changes occur as a consequence of increased hepatic expression of the LDL receptor, which lead to the LDL cholesterol catabolism while stimulating the hepatic production of triglyceride-rich VLDL (Sacks *et al.,* 1994, Leung *et al.,* 2004). Endrikat *et al.* (2002) reported that oral contraceptives containing ethinylestradiol and levanorgestrel caused decrease in HDL2 cholesterol and increase in total cholesterol, LDL cholesterol, VLDL cholesterol and total triglycerides. According to Wiegratz *et al.* (2002) formulations containing ethinylestradiol and levanorgestrel did not change the levels of triglycerides but with a significant increase during treatment with dienogest containing preparations. Although ethinylestradiol and levanorgestrel pills significantly reduced HDL cholesterol and HDL_2 cholesterol, there was a non significant increase with

dienogest containing oral contraceptives. Oral contraceptives containing ethinylestradiol and either desogestrel or cyproterone acetate increased total cholesterol, LDL cholesterol and HDL cholesterol in adolescents with polycystic ovarian syndrome (PCOS) (Mastorakos *et al.*, 2002) and in male Wistar rats (Kamisako and Ogawa. 2003). Koh *et al.* (2003) reported that a combination of conjugated equine estrogen and progesterone significantly reduced levels of total cholesterol and LDL cholesterol while it increased triglyceride and HDL cholesterol in postmenopausal woman.

Estaradiol was found to reduce kidney, knob and channel fat in cattle when administered as ear implants (Enright *et al.*, 1990). Lee *et al.* (1990) observed that estradiol-17*ß* implants in steers reduced fat content of the rib section. Field *et al.* (1990) found no significant effect of estradiol implants on fat deposition in lambs. Pelvic fat depots were found fewer in heifers treated with Zeranol and Estradiol (Moran *et al.*, 1991). Zeranol did not affect back fat thickness over the 12^{th} rib among lambs (Hutcheson *et al.*, 1992). According to Green *et al.* (1992) Estradiol-17*ß* implants inhibited lipogenesis in both lean and obese ewes.

2.1.5 Effect on Lipid oxidation.

Cho *et al.* (1988) observed that estrogen implants in young male chickens caused a two-fold increase in plasma lipid peroxidation measured by the thiobarbituric acid test.

Esterified estrogen reduced lipid peroxidation products to extent of greater than 50 percent in Cynomealous monkeys challenged with an atherogenic diet (Wagner *et al.*, 1996). Zhu *et al.* (1999) observed that when 17ß-estradiol was added to a system containing LDL and incubated alone and combined in the absence or presence of bovine aortic endothelial cells, placental trophoblast, or macrophages an antioxidant effect was observed. Progestins inhibited this protective estrogenic effect. In endothelial cell culture, progestins also opposed the antioxidant effect of estrogen,

with the strongest antiestrogenic effect seen with the synthetic progestins, levonorgestrel and medroxyprogesterone acetate.

2.1.6 Effect on Plasma proteins.

Estradiol increased fibrinogen level in male Wistar rats (Manzano *et al.*, 2002). Levels of total protein and albumin showed a stronger decline after the oral administration of ethinyl estradiol versus transdermal 17β-estrodiol (Giltay *et al.*, 2003).

2.1.7 Effect on enzymes.

Dahlgren *et al.* (1998) observed no derangement of liver enzymes in the women treated with ethinylestradiol and cyproterone acetate.

2.1.8 Effect on Immune system

According to Luster *et al.* (1984) estrogens are known to affect functioning of the immune system. The mechanisms responsible for these effects appear to be complex, mediated through a direct chemical interaction with lymphoid target cells, as well as with nonlymphoid tissue, resulting in the release of soluble immunoregulatory factors. The latter phenomenon appears to constitute a regulatory factor(s) produced by thymic epithelium in response to an estrogen stimulus. The overall augmented humoral immune responses in females and the B cell hyperactivity in female predominant autoimmune diseases appear to be due to estrogen (Ahmed *et al.*, 1989).

Tizard (1996) grouped estrogen and progesterone to nonspecific factors influencing disease resistance. According to him low doses of estrogen stimulate immunity where as high doses of progesterone is immunosuppressive. Administration of estradiol 3-benzoate enhanced significantly the humoral immune response to *Escherichia coli* and sheep erythrocytes (Leitner *et al.*, 1996). Miyaura and Iwata (2002) suggested that progesterone directly or indirectly affects T cell differentiation either by directly inhibiting T helper one development or by enhancing T helper two developments.

2.1.9 Effect on Cardiovascular system.

Nirmalan and Robinson (1972) observed that exogenous oestrogen analogue (Stilbestrol) treated quails had significantly lower erythrocyte count, packed cell volume and hemoglobin concentration. Percentage moisture in metacarpal and metatarsal bone of wethers implanted (sialistic estradiol) for a longer period of time was found to be less and that was resulted from lesser amount of hemopoietic marrow (Field *et al.,* 1990).

Both the coagulation and the fibrinolytic system appeared to have been upregulated by oral contraceptives containing ethinylestradiol and levanorgestrel as it increases pro-coagulatory variables fibrinogen, factor VII Ag, factor VIIa and reduced factor VII act, factor VIII while it reduced anti-coagulatory variables AT III and PCAT slightly (Endrikat *et al.,* 2002). A number of studies have suggested that risk of venous thromboembolism is attributable to the estrogen component in oral contraceptives and that the risk increases in dose dependent manner (Winkler, 1999, Vandenbrouk, *et al.,* 2001, Schindler, 2003).

2.1.10 Effect on organs.

Both zeranol and double implants of estradiol caused precocious mammary gland development in heifers (Moran *et al.,* 1991). Phytoestrogens causes precoccious mammary development in animals, which also cause deleterious effects on ovaries, uterus and other organs. It causes enlargement of bulbourethral glands in wethers and even death (Adams. 1995). Sadek *et al.* (1995) have concluded that broiler chicken fed with oral contraceptives (ethinyl estradiol 88.2 mg and norethisterone acetate 1.764 g /100 kg of feed) developed ultra structural changes in hepatocytes similar to those observed in tumor cells.

Rumsey *et al.* (1996) observed that proportional weight of liver was less in the steers implanted with Synovex-S® (Estradiol benzoate and Trenbolone acetate). Hutcheson *et al.* (1997) noted that liver of steers receiving estrogen implants was

heavier than the control. They also found that liver from androgen plus estrogen treated steers were the heaviest among the different groups.

Liver of estrogen exposed fish have been reported to be enlarged, have hole like lesions, accumulate amorphous eosinophilic material, exibit changes in hepatocytes vacuolization, have less basophilic hepatocytes, or simply resemble the liver of vitellogenic female with increased basophilia and enlarged nuclei (Wester and Canton. 1986, Hamazaki *et al.,* 1987, Herman and Kinacid. 1988, Gimeno *et al.,* 1998a,b; Dreze, *et al.,* 2000. Lange *et al.,* 2001, Metcalfe *et al.,* 2001, Zillioux *et al.,* 2001, Andersen *et al.* 2003 and Weber *et al.,* 2003). The number of hepatocytes with pyknotic nuclei was significantly increased in estrogen exposed zebra fish. There was also a significant increase in the number of hepatocytes that had cloudy swelling.

Ethinylestradiol showed cholestatic effect in rats (Davis and Kern., 1976; Rodriquez *et al.,* 1992; Bossard *et al.,* 1993; Puglielli *et al.,* 1994; Simon *et al.,* 1996; Geier *et al.,* 2003).

In experimental animals prenatal exposure to Diethylstilbestrol (DES) causes urogenital malfunctions, accelerates vaginal openings, persistent estrus, and abnormalities of uterine glands and luminal epithelium in female rat offspring (Rothschild *et al.,* 1988; Biegel *et al.,* 1998; Masutomi *et al.,* 2004).

Administration of estradiol 3-benzoate inhibited comb and testicle growth in chicken (Leitner *et al.,* 1996). Disturbances in steroid hormone profile has the potential to disrupt normal physiology at a number of levels and the potential for endocrine disruption was greatest during the early stages of an animals life (Dawson. 1998). Bortolotti *et al.* (2003) observed that during breeding and winter polychlorinated biphenyl (PCB) exposed kestrels differed from control for both colour and carotenoids. Sexual dimorphism was apparent in colour and carotenoids of control adults, but not for PCB exposed birds. Endocrine disruption was confirmed by depressed level of corticosterone, T_3 and T_4 in exposed birds. Oral

contraceptives containing ethinylestradiol and levanorgestrel/dienogest/ estradiol valerate caused reduction in the serum concentration of testosterone while these increased the level of sex hormone binding globulin and corticosteroid binding globulin level (Wiegratz *et al.,* 2003). These contraceptives also increased T_3, T_4 and cortisol levels in treated individuals (Kuhl *et al.*, 1985 and 1993, Scot *et al.,* 1990, Wiegratz *et al.,* 1995 and Wiegratz *et al.,* 2003). Dietary Genistein increased the vasopressin level in rats (Scallet *et al.,* 2003).

Studies involving administration of steroid hormones revealed increase in tumor growth (Dao *et al.,* 1982, Conte *et al.,* 1985). The most convincing evidence of association of estrogen with the breast cancer is the finding that diethylstilbesterol (DES) caused clear cell adeno-carcinoma of vagina and cervix in daughters of mothers exposed to high DES levels (Greenberg *et al.,* 1984). Rosenblatt *et al.* (1992) found that oral contraceptive use decreased the incidence of ovarian cancer. There was also a report of an increase in the incidence of breast cancer in the mothers exposed to DES (Colton *et al.,* 1993).

From case control studies Toppari *et al.* (1996) suggested that exogenous hormone exposure during pregnancy is associated with increased risk of male offspring developing testicular cancer in adulthood. Estradiol stimulates cell division in hormonally sensitive tissues there by increasing the possibility for accumulation of random errors during DNA duplication. This increased cell proliferation also had the effect of stimulating growth of mutant cells (Henderson and Feigelson. 2000).

Scientific committee on veterinary measures relating to public health of the European commission (1999) indicated that estradiol is carcinogenic to rodents and primarily affect the reproductive organs. However, an increased incidence of bone, pituitary and lymphoid tumors has also accompanied high doses of estradiol in some rodents.

The 1999 review by the UK's subgroup of the veterinary products committee critically evaluated genotoxicity studies of estradiol and concluded that there is no substantial evidence to prove mutagenic/genotoxic effects of estradiol.

Progesterone inhibited myometrial cell proliferation while weakly estrogenic polychlorinated biphenyls stimulated the proliferation in grey seals (Kawaguchi *et al.,* 1985; Shimomura, *et al.,* 1998; Backlin *et al.,* 2003).

The recent pooled analysis of epidemiological studies, focusing primarily on oestrogen replacement therapy, demonstrated a moderate increase in risk of breast cancer with increasing duration of use (Collaborative group., 1997). Other large and well performed studies did not arrive at the same conclusion. In one such study, after 6 years of follow-up, no statistically significant increase in the risk of breast cancer could be detected in "ever users" or "current" users of hormone replace therapy (Folsom *et al.,* 1995; Ameller *et al.,* 2004).

Cho *et al.* (1988) observed that estrogen induced hyperlipidemia resulted in changes in the fatty acid composition of membrane lipids of erythrocytes. The major changes were an increase in oleic acid from 10.0 percent to 14.2 percent and a decrease in linoleic acid from 31.3 percent to 26.0 percent. The erythrocytes with an altered membrane fatty acid composition were found to have an increased osmotic fragility.

2.1.11 Effect on mineral metabolism

Oestrogen substitution diminishes or stops the loss of bone mass in a significantly more protective way than by increasing the supply of calcium or by adding Vitamin D to the diets in postmenopausal women (Cauley *et al.,* 1995; Writing group., 2002; Thijssen., 2003).

Progestins do not add or subtract much of the protective action of estrogens on the bones (Doren and Samsioe., 2000; Banks, *et al.,* 2001; Thijssen., 2003).

2.1.12 Biomarkers for estrogenic exposure.

The circulating yolk precursor protein, vitellogenin, has freequently been used as the biomarker for estrogenic exposure (Sumpter and Jobling. 1995, Korsgaard *et al.*, 2002; Jobling *et al.*, 2004). The hepatic synthesis of vitellogenin may be induced in males as well as in immature fish by estrogenic exposure. Dose dependent increase in vitellogenin production has been observed in zebrafish exposed to ethinylestradiol (Orn *et al.*, 2003).

2.2 EFFECT OF SUPPLEMENTATION OF VITAMIN E IN BROILER CHICKEN

Vitamin E (a-tocopherol) is a biological antioxidant, soluble in fat, which inhibits the oxidation of long chained unsaturated fatty acids of the cell membrane. Unsaturated fatty acids react with oxygen, and form superoxide, peroxide and hydroperoxides. These free radicals cause cell damage by disturbing the metabolism and structure of the biological membranes of those organs that contain excessive amount of unsaturated fatty acids. Vitamin E inhibits the effect of hydrogen protons and free radicals by saturating them, and so inhibits autooxidation.

2.2.1 Effect on Growth

Supplementation of α-tocopherol significantly improved growth in broiler chicken (Lin *et al.*, 1989). High dietary vitamin E did not alter overall growth relative to the basal diet. No differences were apparent for initial weight, average daily gain during the starter or grower phases of pigs fed corn-soybean meal based diets (Lepine *et al.*, 1990). The addition of α -tocopheryl acetate had no effect on average daily gain of pigs (Dove and Ewan. 1991; Anderson *et al.*, 1995).

Feeding lambs with 500 IU of vitamin E over a period of 56 days improved daily gain (Wulf *et al.*, 1995). Liu *et al.* (1995) recommended that feeding 100 to 200 IU vitamin E per steer daily increased weight gain, especially when cattle are stressed. Cannon *et al.* (1996) observed no effect of vitamin E at 100 mg on growth, carcass characteristics such as dressing percentage and shrinkage and proximate composition. Vitamin E supplementation showed no effect on average daily gain in

steers while there was a quadratic effect on average daily gain in heifers (Rivera *et al.*, 2002). Heifers also had a higher dressing percentage.

Weight gain of chicks during 0 – 3 weeks of age was not affected by dietary vitamin E levels of 10 to 20 mg/kg (Hsieh *et al.*, 2002). Live weight gain increased in birds linearly in birds as the dietary vitamin E increased up to 250 mg/kg and further increase up to 500 mg/kg did not increase feed intake (Sahin *et al.*, 2002). Vitamin E at level of 250 mg/kg significantly improved final bodyweight between the supplemented group and control in cold stressed layers (Kucuk *et al.*, 2003).

Al-Taleb (2003) observed that birds fed the diet supplemented with 2.5ml/l vitamin AD_3E plus 15 g vitamin C were significantly ($P<0.05$) heavier than the rest of the birds.

2.2.2 Effect on feed consumption

Kennedy *et al.* (1992) reported that higher levels of vitamin E supplementation could enhance productivity as a result of improvement in both feed conversion efficiency and higher body weight gain in the broiler chicken. Feed intake and gain:feed ratio of chicks during 0 – 3 weeks of age were not affected by dietary vitamin E levels of 10 to 20 mg/kg (Hsieh *et al.*, 2002). Feed intake as well as feed efficiency increased in birds subjected to heat stress linearly as the dietary vitamin E increased up to 250 mg/kg and further increase up to 500 mg/kg did not result in increase in feed intake and feed efficiency (Sahin *et al.*, 2002). Kucuk *et al.* (2003) noted significant improvement in feed efficiency between the Vitamin E (250 mg/kg) supplemented group and control in cold stressed layers. Al-Taleb (2003) observed that the birds fed the diets supplemented with vitamins AD_3E alone or with added vitamin C had a significantly ($P<0.05$) better feed conversion ratio than the control birds.

There were no apparent differences in average daily feed intake or gain/feed during the starter or grower phases of pigs supplemented with Vitamin E (Lepine *et al.*, 1990). The addition of alphatocopheryl acetate had no effect on average daily

feed intake, or the gain:feed ratio of pigs (Dove and Ewan. 1991). Cannon *et al.*, (1996) observed no effect of vitamin E at 100 mg on feeding characteristics in pigs. Average feed intake was not affected while gain:feed ratio increased by supplemental Vitamin E (Soler –Velasquez *et al.*, 1998). According to Waylan *et al.* (2002) supplementing diets with vitamin E in swine did not improve average daily feed intake or gain:feed ratio. However, Hasty *et al.*, (2002) reported that vitamin E (12 to 350 IU per kg) increased average daily feed intake linearly ($P<0.05$) in pigs.

2.2.3 Plasma and tissue profile of Vitamin E

Serum and tissue α-tocopherol increased linearly with increasing dietary α-tocopherol supplementation in swine (Anderson *et al.*, 1995; Moreira and Mahan, 2002). Hill *et al.* (1999) reported that dietary vitamin E supplementation did not show any effect on the plasma vitamin E concentration in pigs. In birds supplemented with vitamin E the plasma vitamin E level of birds in the seventh week of the study increased statistically when compared to the control group (Arslan *et al.*, 2001). Supplementation of broilers with 100 mg kg^{-1} α-tocopheryl acetate significantly increased the vitamin E level in raw breast samples (Carreras *et al.*, 2004).

2.2.4 Effect on lipid profile

Franchini *et al.*, (1988) reported that the supplementation of vitamin E in broiler chicken at a dosage of 325 ppm resulted in a decrease in cholesterol and triglyceride levels, and the decrease related to age is definite by day 49.Dietary vitamin E supplementation has no significant effect on cholesterol level in broiler chicken (Donaldson 1982; Smith and Kummerow 1989). It has been reported that the cholesterol level of turkeys fed with a vitamin E supplemented diet decreased on the day 42 and it reached its maximum level on the day 86 (Franchini *et al.*, 1990). In the same study, it was found that increasing the levels of vitamin E lowered level of triglycerides on the day 28, and on day 42 it increased the level of triglyceride. Vitamin E supplementation substantially exceeding levels normally considered

adequate does not lower the total cholesterol concentration of pigs fed corn-soybean meal based diets (Lepine *et al.*, 1990). Wuryastuti *et al.* (1993) and Soler –Velasquez *et al.* (1998) reported that there was no association between serum tocopherol and cholesterol concentrations and vitamin E is unevenly distributed between serum and lipoprotein components such as the low and high density lipoprotein fractions in sows.

Arslan *et al.* (2001) observed no significant difference in the plasma concentration of cholesterol and triglycerides of control and vitamin E supplemented birds by the fifth and seventh week of age. In weanling pigs vitamin E at levels of 20, 40, 60 and 100 IU/kg diet had no effect on serum triglyceride concentration (Moreira and Mahan, 2002).

Sahin *et al.* (2002) reported that, an increase in dietary vitamin E linearly reduced serum triglycerides and cholesterol concentration in birds exposed to heat stress. Kucuk *et al.* (2003) observed significant reduction in serum triglycerides and cholesterol concentrations in cold stressed layers supplemented with 250mg/kg vitamin E. Intramuscular vitamin E administration at level of 200 IU/animal failed to elicit any response in triglycerides, cholesterol levels in serum and total lipids in muscles of cross bred lambs (Salvatori *et al.*, 2004).

2.2.5 Effect on lipid oxidation.

Vitamin E deficiency has been reported to be frequently associated with an increased susceptibility to free radical oxidation (Dormandy, 1978). Polyunsaturated fatty acids (PUFA) of membranes are particularly vulnerable to attack by reactive oxygen species (ROS), and ROS can initiate a chain reaction of lipid destruction that destroys the membrane of the cell. Vitamin E can quench peroxidation reactions in membranes and is probably the most important antioxidant located in cell membranes (Putnam and Comben, 1987). Cho *et al.* (1988) observed that vitamin E supplementation (1,000 IU/kg diet) reduced the plasma lipid peroxidation to the control level, but had no effect on the plasma lipid content in young male chicken.

The common function that underpins the diverse applications of vitamin E is mainly its ability to function as an antioxidant in biological systems. Free radicals are neutralized by α-tocopherol before lipid oxidation propagates among highly unsaturated fatty acids in cellular and subcellular membranes (Burton and Ingold, 1989; Hogan *et al.,* 1993;1996). The chromanol ring of a-tocopherol is located among the polar head groups of the phospholipids, and the phytol side chain interacts with the unsaturated fatty acyl chains of the phospholipids through van der Waals interactions in the interior of the membrane (Gomez-Fernandez *et al.,* 1989; Kagan, 1989). This specific localization of α-tocopherol in the membrane and the molecule's lateral mobility allow it to function very efficiently to protect highly oxidizable polyunsaturated fatty acids from peroxidation by reactive oxygen species produced by adjacent membrane-bound enzymes (Gomez-Fernandez et al., 1989 and Liu *et al.,* 1995).

Packer (1991) opined that dietary vitamin E supplementation can provide protection from free radical damage, in lambs (Wulf *et al.,* 1995 and Salvatori *et al.,* 2004) and in pigs (Cannon *et al.,* 1996). It is now being realized that one of the reasons for majority of toxicities/ disorders is imbalance between amounts of free radicals generated in the body and antioxidants to scavenge and protect the body against their deleterious effects (Halliwell, 1992).

Oxidant by-products of normal metabolism cause extensive damage to DNA, protein, and lipid. This damage (the same as that produced by radiation) is a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts. Antioxidant defenses against this damage include ascorbate, tocopherol, and carotenoids (Ames *et al.,* 1993).

Ushakova *et al.* (1996) pointed out that dietary supplementation of vitamin E have a protective role against oxidative stress by enhancing the level of endogenous antioxidants in mice. Vitamin E is an integral component of all lipid membranes and

serves to protect lipid membranes from attack by reactive oxygen species (Smith *et al.,* 1997). Lipid oxidation measured as thiobarbituric acid reactive substances (TBARS) concentration was not affected by level of vitamin E supplementation in horses (Siciliano *et al.,* 1997) and in pigs at levels of 100, 200 and 300 mg/kg (Ohene-Adjei *et al.,* 2004).

Dietary supplementation of vitamin E (200 mg/kg of feed) increased the α-tocopherol concentration in muscle and its membranous sub-cellular fractions and protected membranal lipids in broiler chicken (Lauridsen *et al.,* 1997), in lambs (Guidera *et al.,* 1997) and in beef (Mitsumoto *et al.,* 1998 and O'Grady *et al.,* 2001).

Oxidation as indicated by thiobarbituric acid reactive substances (TBARS) decreased linearly ($P<0.001$) with addition of vitamin E in gilts (Hasty *et al.,* 2002). According to Hsieh *et al.* (2002) the oxidative stability in liver was increased by increased dietary vitamin E levels of 10 to 20 mg/kg with out any influence on the oxidative stability of heart and breast muscles. Supplemental vitamin E (250 mg/kg) significantly reduced lipid peroxidation in serum of cold stressed layers (Kucuk *et al.,* 2003). Jaysree *et al.* (2003) reported deltamethrin induced toxicity by oxidative damage in biological system and supplementing vitamin E in feed was effective in the treatment of accidental toxicity in broiler chicken. Vitamin E reduced sensory meat rancidity showed the effectiveness of dietary α-tocopheryl acetate supplementation in protecting broiler meat against lipid oxidation (Carreras *et al.,* 2004).

Cells with added vitamin E exhibited increased viability and accumulated less lipid hydroperoxides than cells without vitamin E supplementation in-vitro (Wrona *et al.,* 2004). Dotan *et al.* (2004) opined that vitamin E failed to show significant correlation with antioxidant enzyme systems while vitamin C did had the effect.

2.2.6 Effect on plasma proteins.

Arslan *et al.* (2001) found no significant difference in total protein between control and vitamin E supplemented birds by the fifth and seventh weeks of age Serum concentration of total proteins and albumin increased as dietary vitamin E increased up to 250 mg/kg diet, but further increase up to 500 mg/kg diet failed to elicit any response (Sahin *et al.*, 2002).

2.2.7 Effect on plasma and tissue enzymes

Franchini *et al.*, (1988) investigated the effect of vitamin E on enzyme activity in broilers and they did not observe any change between the control and experimental groups with respect to aspartate aminotransferase (AST) activity on the 49th day of the study. Franchini *et al.*, (1990) reported that the AST level increased together with vitamin E in young turkeys, although a decrease was observed in older turkeys (140 days old), and these results were dissimilar to those observed in hens.

Arslan *et al.* (2001) observed no significant difference in plasma alkaline phosphatase (ALP), (AST) or alanin aminotransferase (ALT) between control and vitamin E (100, 200 and 300 ppm) supplemented birds by the fifth and seventh weeks.

Serum activities of AST and ALT were not influenced by dietary vitamin E supplementation in birds subjected to heat stress (Sahin *et al.*, 2002). However serum activity of ALP increased linearly with increasing dietary vitamin E supplementation.

In muscles of calves, depleted vitamin E, Walsh *et al.* (1993) observed that vitamin E did not control superoxide desmutase (SOD) activity. Renerre *et al.*, (1999) observed that vitamin E supplementation had no effect on SOD activity in turkey meat. Gatellier *et al.* (2004) noticed in cattle a highly significant ($p < 0:001$) correlation ($r = 0:591$) between SOD activity and vitamin E content of muscles.

2.2.8 Effect on Immune system

Dietary supplementation of vitamin E is beneficial to the overall immunocompetence of growing broilers (Erf *et al.*, 1997). Subcutaneous injections

of vitamin E approximately 10 and 5 days before calving successfully elevated polymorpho nuclear cells during the periparturient period and reduced the incidence of intramammary infections in cattle (Smith *et al.*, 1997).

Reffet *et al.* (1988) and Daniels *et al.* (2000) did not find any effect of vitamin E on Immunoglobulin G (IgG) levels of lambs. According to Tengerdy (1989) and Field *et al.* (2002) vitamin E as a dietary supplement or as part of an adjuvant vaccine formulation increase humoral and cell-mediated immunity with disease resistance in laboratory animals, farm animals, and humans. The most pronounced effect of vitamin E is on immune phagocytosis. Vitamin E plays an important role in phagocytosis involving polymorphonuclear cells in sows (Wuryastuti *et al.*, 1993) and cattle (Hogan *et al.*, 1993 and Smith *et al.*, 1997).

Rivera *et al.*, (2002) observed an increase in circulating antibodies to foreign antigen with supplementation of 1140 IU/d indicated that vitamin E can enhance humoral immune response in cattle. Vitamin E supplementation in yearling ewes (0 to 330 IU per ewe per day) showed no influence on humoral immune response (Hatfield *et al.*, 2002).

2.2.9 Effect on organs.

According to Upasani and Balaraman (2001) Vitamin E had ability to restore the normal levels of lipids in the liver, lung, heart and kidney of rats exposed to the peroxidative damage of free radicals induced by lead. The oxidative stability in liver was increased by increased dietary vitamin E levels of 10 to 20 mg/kg while there was no influence on the oxidative stability of heart musculature (Hsieh *et al.*, 2002).

Vitamin E did not affect Sertoli or germ cell population at various ages. Boars at 18 months of age had lower PGF2α concentrations in the prostate and seminal vesicles when vitamin E was fed. Dietary vitamin E, however, had no effect on sperm number at any stage of development (Marin-Guzman *et al.*, 2000).

Vitamin E protects *Escherichia coli* cells from oxidative mutagenesis. Vitamin E achives this by preventing DNA adducts formation by lipid peroxidation

products (Nikolic *et al.*, 2004). Vitamin E pretreatment resulted in significant decline of aberrant metaphases and chromosomal aberrations induced by known hepatocarcinogen *N*-Nitrosomorpholine in HepG2 cell lines (Robichova *et al.*, 2004). Choi *et al.* (2004) observed no evidence of either a protective or deleterious effects on DNA damage, resistance of DNA to oxidant challenge or lipid peroxidation to acute single dose of vitamin E (400 IU) in healthy human beings. Tocopherols (0.1 to 10 μM) inhibited cyclooxygenase-2 activity in stimulated colon cancer cells and thus emphasizing the anticarcinogenic property (O'Leary *et al.*, 2004). Vitamin E did not significantly affect the incidence of gastric, pancreatic, colorectal and hepatocellular carcinoma in humans (Bjelakovic *et al.*, 2004). They also reported that the mortality rate was increased in people supplemented with antioxidants.

Erythrocyte osmotic fragility of experimental birds supplemented with vitamin E decreased significantly by the fifth and seventh week of the study in comparison with the control group (Bieri *et al.*, 1976; Levander *et al.*, 1977 and Arslan *et al.*, 2001).

Lauridsen *et al.*, (1999) reported that pigs supplemented with dl-a-tocopheryl acetate showed significantly higher water holding capacity indicating the membrane-stabilizing effect of vitamin E.

Vitamin E stabilizes the membrane of immune cells in rats fed the high vitamin E (500 IU α-tocopherol nicotinate/kg) diet for seven weeks (Moriguchi and Itoh, 1997; Kaminogawa and Nanno, 2004).

2.2.10 Effect on mineral metabolism

Arslan *et al.* (2001) observed no significant difference in plasma calcium and phosphorus levels in vitamin E supplemented birds by the fifth and seventh week of age. Increasing dietary vitamin E caused linear increase in serum concentration of calcium and phosphorus (Sahin *et al.*, 2002). According to Kucuk *et al.*, (2003) serum concentrations of calcium and phosphorous increased upon supplementation of vitamin E at a level of 250 mg/kg in cold stressed layers.

2.2.11 Biomarkers for oxidative dammage.

Reports of Dawn-Linsley *et al.* (2004) supported the use of thiobarbituric acid reactive substances (TBARS) assay for the analysis of oxidative damage in nervous tissue and in cultured neurons. Dotan *et al.* (2004) opined that the commonly used criteria based on lipid peroxidation can not be regarded as a general estimate of the individual oxidative status. In their review they also revealed that only under severe pathological conditions (like AIDS) all the indices of oxidative stress correlate with each other.

Lim *et al.* (2004) suggested that serum γ-glutamyltransferase (GGT) level within normal range may act as an early marker of oxidative stress in humans. They also reported that vitamin E was not associated with serum GGT level.

Materials and Methods

3. MATERIALS AND METHODS

The experiment was conducted in commercial broiler chicken (Vencob strain) in the Department of Physiology, College of Veterinary and Animal Sciences, Mannuthy for a period of four weeks from fourth to eighth week of age to study the effect of dietary supplementation of synthetic steroid hormones and α-tocopherol in broiler chicken.

3.1 EXPERIMENTAL BIRDS.

Thirty-two, healthy day old commercial broiler chicks (Vencob strain) were procured from commercial hatchery. Birds were numbered and divided into four groups viz G I, G II, G III and G IV (eight birds in each group) and reared for four weeks in cage system under standard management conditions with standard broiler ration as per BIS (1992). From fourth to eighth week of age, birds of different groups were fed with broiler ration with or without steroid hormones and α-tocopherol as follows:

G I (control) - Standard broiler ration.

G II - Standard broiler ration + Ethinylestradiol and Levanorgestrel incorporated @ 66.3 mg & 331.5 mg respectively per 100 kg of feed.

G III- Standard broiler ration + dl-α-tocopherol (25 g per 100 kg of feed).

G IV- Standard broiler ration + dl-α-tocopherol and a combination of Ethinylestradiol and Levanorgestrel at 66.3 mg & 331.5 mg respectively per 100 kg of feed.

3.2 EXPERIMENTAL RATIONS.

The standard broiler ration (starter and finisher) formulated as per Bureau of Indian Standards specifications (BIS, 1992) vide table 1, and proximate principle was estimated as per Association of Official Analytical Chemists (AOAC, 1990) (table 2). Day old chicks were fed with broiler starter ration upto fourth week of age. From fourth week of age to eighth week birds were fed with finisher ration.

Table 1. Composition of broiler chicken ration

Sl. No.	Ingredients	Standard broiler ration (kg)	
		Starter	Finisher
1.	Yellow maize	44.00	54.00
2.	Ground nut cake	27.00	19.00
3.	Soyabean meal	6.00	7.00
4.	Gingelly oil cake	3.00	Nill
5.	Unsalted dried fish	8.00	7.00
6.	Rice polish	10.00	11.00
7.	Common salt	0.25	0.25
8.	Mineral mixture *	1.75	1.75
	Total	100.00	100.00
Added per 100 kg of feed			
9.	Vitamin mixture (g)**	15.00	15.00
10.	Lysine hydrochloride (g)	200.00	200.00
11.	Methionine (g)	100.00	
12.	Coccidiostat (g)***	50.00	50.00
13.	Manganese sulphate	10.00	10.00

* Keyes Mineral mixture® (M/s Kerala Solvent Extractions Limited, Irinjalakuda, Kerala, India): Calcium 32%, Phosphorus 6%, Magnesium 1000 ppm, Cobalt 60 ppm, Zinc 2600 ppm, Iron 0.1%, Iodine 100 ppm, Copper 100 ppm and Manganese 2700 ppm.

** Indomix® (M/s Nicholas Piramal India Limited, Mumbai, India): Each gram contains:- Vit. A – 82,500 IU, Vit. B_2 – 50 mg, Vit. D_3 – 12,000 IU and Vit. K – 10 mg.

*** Anacox® (M/s Trends Pharma Private Limited, Rajpipla, Gujrat, India, India): Each gram contains Madhuramicin ammonium 1% w/w.

Table 2. Proximate principle of broiler chicken ration (on dry matter basis).

Sl. No.	Ingredients	Standard broiler ration (kg)	
		Starter	Finisher
Analysed values*			
1.	Moisture	9.6	9.48
2.	Crude protein	23.54	20.35
3.	Ether extract	5.87	5.95
4.	Crude fibre	5.28	4.96
5.	Nitrogen free extract	54.01	57.32
6.	Total ash	11.30	11.42
7.	Acid insoluble ash	2.46	2.50
8.	Calcium	1.40	1.34
9.	Phosphorus	0.80	0.73
Calculated values			
10.	Metabolizable energy (kcal/kg)	2802.00	2904.00
11.	Lysine (%)	1.50	1.00
12.	Methionine (%)	0.53	0.40
13.	Manganese (mg/kg)	104.00	102.00

* Mean of eight samples.

3.3 EVALUATION OF HEALTH STATUS AND BODY WEIGHT OF BIRDS.

The birds were daily evaluated for their health status. The initial and weekly body weight of birds in each group was recorded till eighth week of age. Average daily body weight gain was calculated using the data.

3.4 BLOOD COLLECTION.

Blood samples (5 ml each) were collected with and without anticoagulants (heparin and EDTA) from wing vein viz fourth week, sixth week and eighth week (at the end of experiment) and used for hematological and biochemical evaluations.

Blood samples collected with anticoagulant (Heparin 20 IU/ml of blood and EDTA 1 mg/ml) were subjected to the estimation of various haematological parameters. Plasma was separated from blood samples by centrifuging at 3000 r.p.m. for 30 minutes, aliquoted and stored at -20°C till further analysis. Serum was separated from blood samples collected without anticoagulants, aliquated and stored at -20°C till further analysis of biochemical constituents and minerals. The activity of plasma enzymes were measured on the day of blood collection.

3.5 ESTIMATION OF HAEMATOLOGICAL PARAMETERS.

Total erythrocyte count (TEC) and total leucocyte count (TLC) were estimated by the method suggested by Natt and Herric (1952) while Volume of packed red blood corpuscles (VPRC) was determined by micro haematocrit method (Feldman *et al.,* 2000) on the day of blood collection. The concentration of haemoglobin (Hb) was estimated by acid hematin method as described by Feldman *et al.* (2000).

Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Swenson and Reece, 1996).

3.6 ESTIMATION OF BLOOD BIOCHEMICAL PARAMETERS.

3.6.1 Protein profile.

3.6.1.1 Total protein: Plasma concentration of total proteins was estimated by Biuret method, as suggested by Henry *et al.* (1957) using Ecoline® Kit (M/s E. Merk, India, Limited, Mumbai).

3.6.1.2 Albumin: Concentration of plasma albumin was estimated by Doumas method as described by Doumas *et al.* (1971) using Ecoline® Kit (M/s E. Merk, India, Limited, Mumbai).

3.6.1.3 Globulin: The plasma globulin content was determined by subtracting plasma albumin level from the total plasma protein content

3.6.1.4 A:G ratio: A:G ratio was calculated from the values of albumin and globulin obtained.

3.6.2 Lipid profile.

3.6.2.1 Total lipids: Concentration of total plasma lipids was estimated by phosphovainilline method, as described by Chabrol (1961) using Labkit® Kit (M/s Labkit, Spain).

3.6.2.2 Triglycerides: Concentration of plasma triglycerides was estimated by the method of Schettler and Nussel (1975) using Ecoline® Kit (M/s E. Merk, India, Limited, Mumbai).

3.6.2.3 Total cholesterol: The concentration of total plasma cholesterol was estimated by cholesterol phenol aminoantipyrine (CHOD-PAP) method as suggested by Richmond (1973) using Ecoline® Kit (M/s E. Merk, India, Limited, Mumbai).

3.6.2.4 High density lipoprotein (HDL) cholesterol: The HDL cholesterol concentration was estimated by precipitation method using phosphotungstate-magnesium chloride (Bachorik *et al.*, 1976) using kit from Agappe Diagnostis, Maharashtra.

3.6.2.5 Low density lipoprotein (LDL) cholesterol: The concentration of LDL cholesterol was calculated using the equation suggested by Mastorakos *et al.* (2002).

LDL cholesterol = Total cholesterol – (Triglycerides / 5) – HDL cholesterol.

3.6.2.6 Very low density lipoprotein (VLDL) cholesterol: The concentration of VLDL cholesterol was derived using the Friedewald equation (Friedewald *et al.*, 1972)

$$\text{VLDL cholesterol} = \frac{\text{Triglycerides}}{5}$$

3.6.3 Activity of plasma enzymes

3.6.3.1 Gamma glutamyltransferase (GGT)

The plasma gamma glutamyltransferase (GGT) level was estimated using the p-Nitroaniline method developed by Szasz (1974) using Ecoline® Kit (M/s E. Merk, India, Limited, Mumbai).

3.6.3.2 Aspartate aminotransferase (AST)

The activity of plasma aspartate aminotransferase (AST) of the sample was estimated by UV kinetic method (Bergmeyer, 1974) using Ecoline® Kit (M/s E. Merk, India, Limited, Mumbai).

3.6.3.3 Superoxide dismutase (SOD)

The activity of superoxide dismutase (SOD) of the sample was estimated by method suggested by Winterbourn *et al.* (1975).

Principle

The activity was measured based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium by superoxide.

Reagents used

1. Phosphate buffer (PBS); 0.06 M; pH 7.8

A. Dissolved 0.936 g $NaH_2PO_4.2H_2O$ in 100 ml double distilled water.

B. Dissolved 0.95 g Na_2HPO_4 in 100 ml of double distilled water.

C. Mixed 8.5 ml of A and 91.5 ml of B. This was done by slowly adding A to B checking pH periodically; once pH of 7.8 is attained, addition of A was stopped.

2. EDTA and Sodium cyanide (NaCN) solution.

Sodium cyanide solution was prepared by dissloving 2g NaCN in 1 ml of double distilled water. Weighed 3.72 g of EDTA and transferred it to a 100 ml volumetric flask and mixed by adding 60 ml double distilled water and finally made up the volume to 100 ml using double distilled water. Dissolved 150 µl of NaCN in 100 ml of 0.1 M EDTA.

3. Nitro blue tetrasolium (NBT)

Weighed accurately 12.3 mg of NBT and added it to 10 ml of PBS buffer.

4. Riboflavin (2 µM solution)

Dissolved 4.5 mg riboflavin in 100 ml of PBS buffer.

Sample preparation

Took 100 µl of blood with anticoagulant and added 900 µl of cold distilled water at 4°C. Added 0.25 ml chloroform and 0.5 ml ethanol with vigorous mixing. Centrifuged the mixture at 18,000 rpm for 60 mintues under refrigeration. The clear supernatant was used for SOD assay at 560 nm using spectrophotometer.

Assay

	Supernatant µl	PBS ml	EDTA-NaCN µl	NBT µl	Riboflvin µl
Standard		2.650	200	100	50
Test	100	2.550	200	100	50

Kept it for 15 minutes under 40 w fluorescent bulb illumination and the end OD read at 560 nm in a spectrophotometer.

Calculation

$$\text{Percentage of inhibition} = \frac{\text{O.D. of standard} - \text{O.D. of test}}{\text{O.D. of standard}} \times 100$$

Volume of supernatant

having 50 percentage inhibition, $A = \frac{100}{\%\ \text{inhibition}} \times 50$

Amount of haemoglobin (Hb) in A, $B = \frac{A}{1750} \times$ Hb (in g percent of blood)

One SOD unit, $C = \frac{1}{B}$

SOD per g of haemoglobin $= C \times 1000$ units

3.6.3.4 Catalase

Blood catalase activity was estimated by following the method suggested by Aebi (1974).

Reagents used

1. Phosphate buffer (PBS) 50mM, pH 7

A. Dissolved 1.7 g KH_2PO_4 in double distilled water; made upto 250 ml.

B. Dissolved 4.45 g of $Na_2HPO_4.2H_2O$ in 500 ml double distiled water.

C. Mixed 250 ml of A and 387.5 ml of B and stored at 4°C.

2. Hydrogen peroxide (H_2O_2) fresh; 30mM

Diluted 0.34 ml of H_2O_2 with 100 ml phosphate buffer and stored at 2°C.

Preparation of sample

After separating the plasma sediment of erythrocytes were washed with normal saline three times. To 200 µl of the thick sediment added 4 or 5 parts of distilled water so as to get a stock solution containing 5 g% of haemoglobin. To 10 µl of this haemolysate added 5 ml of phosphate buffer.

Assay

Wavelength : 240 nm (UV range)

	PBS ml	Haemolysate ml	H_2O_2 ml
Standard	1	2	-
Test	-	2	1

Recorded the initial O.D. and O.D. at every 10 seconds for 2 minutes using spectrophotometer at 240 nm. From the initial and the final O.D, catalase activity was found out using the following formula.

$$\text{Catalase (k / g of Hb)} = \frac{2303}{T} \times \log \frac{\text{First reading}}{\text{Second reading}} \times \frac{a}{b} \times 1000$$

Where T – time interval = 10 seconds
a – Constant = 1250
b – = haemoglobin concentration X 10

3.6.3.5 Plasma lipid peroxidation level

Plasma lipid peroxidation level was found out by melon-di-aldehyde method as described by Ohkawa *et al.* (1979).

Reagents used

1. 20% Trichloro acetic acid (TCA)
2. 0.67% Thiobarbituric acid (TBA)

Took 0.67 g of TBA and added 100 ml of distilled water and heated slightly to dissolve the content completely without boiling.

3. n-Butanol.

Assay

Took 0.5 ml of plasma; added 2.5 ml 20% TCA and 1 ml of 0.67 % TBA. Mixed well and kept it for 30 minutes in boiling water bath. Cooled under running tap water. Added 4 ml n-Butanol and mixed well. Centrifuged at 1000 rpm for 5 minutes to separate organic layer. Read absorbance of supernatant at 353 nm against blank (n-butanol) in a spectrophotometer. Peroxidation level was found out using standard calibration curve constructed using different known standard solutions (2, 4, 6, and 8 n mol/ml).

3.6.4 Estimation of minerals in serum

The serum profile of minerals like calcium, phosphorous, iron and copper were estimated using the method suggested in AOAC guidelines 1990 using atomic absorption spectrophotometer (AAS).

3.7 ESTIMATION OF BIOCHEMICAL PARAMETERS IN TISSUES

3.7.1 Tissue homogenization

Liver and breast muscle samples were weighed (1 g) and homogenized in 0.15 M NaCl (5 ml) for lipid peroxidation and for GGT estimation, and homogenates of 20 % were obtained (One g tissue in five ml of 0.15 M NaCl). Tissue homogenates were sonicated twice at an interval of 30 seconds. Homogenization and sonication were performed at 4°C. After sonication, homogenates for lipid peroxidation and biochemical studies were centrifuged at 3000 rpm for 10 minutes and at 15000 rpm for 15 minutes respectively at 4°C. Aliquots of the supernatants were used for both GGT as well as tissue peroxidation level estimation (Ozaras *et al.*, 2003).

3.7.2 Activity of gamma glutamyltransferase (GGT) in liver and muscle

The tissue gamma glutamyltransferase (GGT) level was estimated using the p-Nitroaniline method developed by Szasz (1974) using Ecoline® Kit (M/s E. Merk, India, Limited, Mumbai).

3.7.3 Tissue peroxidation level (liver and skeletal muscles)

Peroxidation levels of tissues were estimated similar to the one adopted for plasma samples.

Reagents used

1. 20% Trichloro acetic acid (TCA)
2. 0.67% Thiobarbituric acid (TBA)

Took 0.67 g of TBA and added 100 ml of distilled water and heated slightly to dissolve the TBA completely without boiling.

3. n-Butanol.

Assay

Took 0.5 ml of aliquot prepared from tissue samples as per the method suggested by Ozaras *et al.* (2003); added 2.5 ml 20% TCA and 1 ml of 0.67 % TBA. Mixed well and kept it for 30 minutes in boiling water bath. Cooled under running tap water. Added 4 ml n-Butanol and mixed well. Centrifuged at 1000 rpm for 5

minutes to separate organic layer. Read absorbance of supernatant at 353 nm against blank (n-butanol) in a spectrophotometer. Peroxidation level was found out using standard calibration curve constructed using different known standard solutions (2, 4, 6, and 8 n mol/ml).

3.7.4 Crude protein and Ether extract of skeletal muscles.

The crude protein and ether extract of breast muscles were estimated as per the methods of Association of Official Analytical Chemists (AOAC, 1990).

3.8 HISTOMORPHOLOGY OF LIVER, ADRENAL AND SKELETAL MUSCLES

Representative samples of tissues were collected in 10% Neutral buffered formalin (pH: 7). The tissues were processed by routine paraffin embedding techniques (Sheehan and Hrapchak, 1980). Sections were cut at 4 micron thickness and stained with routine Haematoxylin and Eosin (Bancroft and Cook, 1995) for histological studies. The stained sections were examined in detail under light microscope and the lesions were classified.

3.9 STATISTICAL ANALYSIS

Data obtained from the experiment were compared by analysis of variance (ANOVA) according to the General Linear Model procedure of SPSS package (SPSS/PC+statistic 10.0 SPSS Inc. Chicago, IL., 2000).

Results

4. RESULTS

4.1 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON PRODUCTION PERFORMANCE OF BROILER CHICKEN

4.1.1 Body weight

The body weight of birds in various groups did not differ significantly through out the experimental period (table 3 and fig. 1). The birds in α-tocopherol supplemented group had slight advantage of around 50 g initially and that was consistently observed in sixth as well as in the eighth week also. Apart from that all treatments failed to elicit significant influence in body weight of experimental birds.

4.1.2 Average daily gain (ADG)

There was significant difference ($P<0.05$) in ADG values in birds fed with steroids (ethinylestradiol+levanorgestrel) and α-tocopherol (G-IV), in the control birds of group G-I and ethinylestradiol+levanorgestrel fed birds of group G-II (table 3). The ADG was highest in birds fed with steroids and α-tocopherol group G-IV (55.87 ± 0.04 g) and lowest in birds fed only with steroids alone group G-II (45.41 ± 0.01 g). Similar values were observed in birds of groups G-III and G-IV, which were higher than the values in both control (G-I) and ethinylestradiol+levanorgestrel (G-II) fed groups.

4.1.3 Feed efficiency (FE)

The feed efficiency value at fourth, sixth and eighth week of age for the four treatment groups are given in table 4.

At fourth week of age there was no significant variation in feed efficiency among the various groups. The lowest feed efficiency (2.00 ± 0.05) was recorded in birds of α-tocopherol supplemented group G-III and highest feed efficiency was observed in birds fed with both ethinylestradiol+levanorgestrel and α-tocopherol fed group G-IV (1.90 ± 0.03).

Table 3. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on body weight, average daily gain (ADG) and dressing percentage of broiler chicken from fourth to eighth week of age, mean ± S.E.(n = 8)

Group	Body weight (g)					ADG (g/day)	Dressing percentage
	Age in weeks						
	Fourth	Fifth	Sixth	Seventh	Eighth		
G-I	899 ± 0.14	1486 ± 0.06	1786 ± 0.09	2091 ± 0.10	2371 ± 0.11	46.05± 0.03[a]	71.73 ± 0.71[a]
G-II	1043 ± 0.07	1514 ± 0.04	1807 ± 0.04	2064 ± 0.04	2314 ± 0.03	45.41 ± 0.01[a]	73.97 ± 0.44[b]
G-III	1093 ± 0.05	1607 ± 0.07	1956 ± 0.07	2286 ± 0.08	2629 ± 0.07	52.81 ± 0.01[ab]	74.47 ± 0.52[b]
G-IV	1029 ± 0.03	1571 ± 0.03	1930 ± 0.06	2276 ± 0.09	2593 ± 0.12	55.87 ± 0.04[b]	71.10 ± 0.49[a]

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b) in columns differ significantly ($P<0.05$).

At sixth week of age also there was no significant difference in feed efficiency among the various groups. The birds of control group G-I had a value of 2.44 ± 0.02 which was comparatively lower than the rest of the birds of groups G-II, G-III and G-IV (2.35 ± 0.04, 2.42 ± 0.05 and 2.37 ± 0.06 respectively).

At eighth week of age too there was no significant variation in feed efficiency of control group G-I when compared with groups of G-II, G-III and G-IV. The birds of group G-IV fed with ethinylestradiol+levanorgestrel and α-tocopherol had poor feed efficiency (3.11 ± 0.18) and α-tocopherol supplemented group G-III had the better (2.92 ± 0.07) feed efficiency.

4.2 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON SLAUGHTER TRAITS

4.2.1 Dressing percentage

Effect of ethinylestradiol+levanorgestrel and α-tocopherol supplementation on dressing percentage in the four treatment groups at slaughter is given in table 3 and fig. 2. Significantly ($P<0.05$) lower values were recorded in control group G-I (71.73 ± 0.71 percent)and birds fed with ethinylestradiol+levanorgestrel and α-tocopherol G-IV (71.10 ± 0.49 percent) when compared to birds in ethinylestradiol+levanorgestrel fed group G-II (73.97 ± 0.44 percent) and α-tocopherol alone fed group G-III (74.47 ± 0.52 percent).

4.3. EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON HAEMATOLOGICAL PARAMETERS

The values of certain haematological parameters such as total erythrocyte count (TEC), haemoglobin (Hb) concentration, total leucocyte count (TLC), volume of packed red cells (VPRC), erythrocyte sedimentation rate (ESR), erythrocyte indices viz., mean corpuscular volume(MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of broiler chicken at fourth, sixth and eighth week of age under the dietary supplementation of ethinylestradiol+levanorgestrel and α-tocopherol are depicted in tables 5a and 5b.

Table 4. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on feed efficiency of broiler chicken from fourth to eighth week of age, mean ± S.E. (n = 8)

Group	Fortnightly mean feed efficiency			Cumulative mean feed efficiency
	Age in weeks			Age in weeks
	fourth	Sixth	Eighth	fourth - Eighth
G-I	1.92 ± 0.05	2.44 ± 0.02	3.08 ± 0.06	2.48 ± 0.13
G-II	1.94 ± 0.05	2.35 ± 0.04	2.97 ± 0.13	2.42 ± 0.18
G-III	2.00 ± 0.05	2.42 ± 0.05	2.92 ± 0.07	2.45 ± 0.08
G-IV	1.90 ± 0.03	2.37 ± 0.06	3.11 ± 0.18	2.46 ± 0.05

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group;
G-III- dl-α-tocopherol acetate fed group and
G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Fig. 1. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-a-tocopherol acetate on the body weight (kg) of broiler chicken from fourth to eighth week of age.

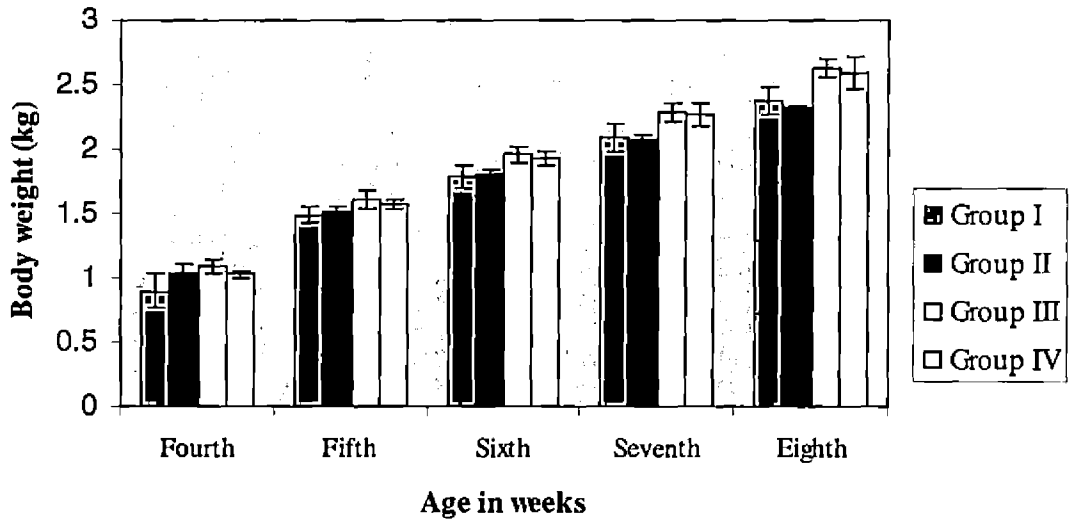
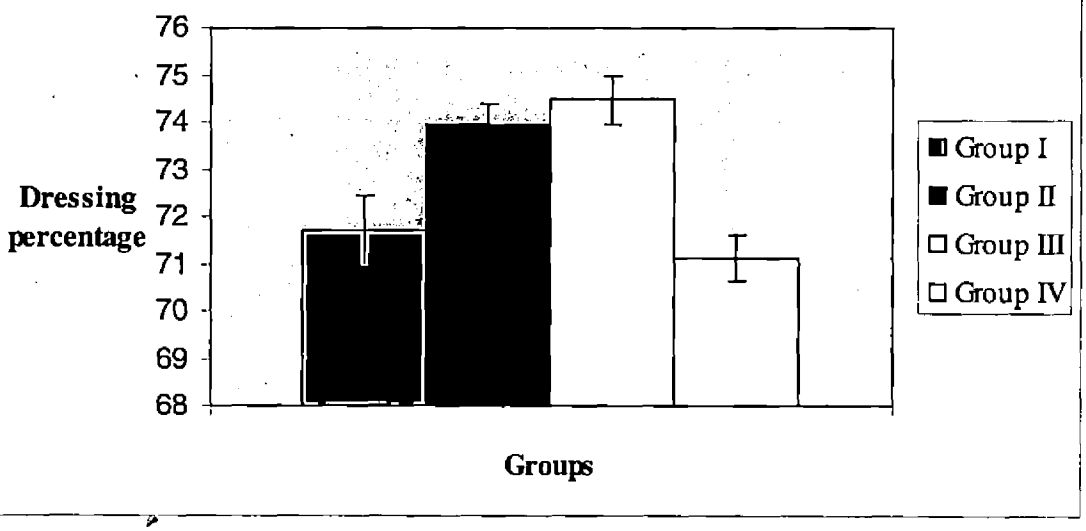


Fig. 2. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on the dressing percentage in broiler chicken



Group -I- Control ; Group-II- Ethinylestradiol+levanorgestrel fed group; Group-III- dl-α-tocopherol acetate fed group and Group-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

4.3.1 Total erythrocyte count (TEC)

There was no significant difference ($P>0.05$) in TEC among various groups (table 5a) at fourth week of age (initial period) with values ranging from $3.39 \pm 0.08 \times 10^6/\mu l$ in birds of G-I group (control) to $3.53 \pm 0.04 \times 10^6/\mu l$ in birds of G-III group (α-tocopherol supplemented group).

At sixth week of age there was significant difference ($P<0.05$) in the values of TEC between birds of G-I group (control) and G-II (birds fed with ethinylestradiol+levanorgestrel), G-III (birds fed with α-tocopherol) whereas TEC values were similar in birds of groups G-I (control) and G-IV birds fed with ethinylestradiol+levanorgestrel and α-tocopherol. The value of G-I (control) was the highest ($3.68 \pm 0.05 \times 10^6/\mu l$) where as, birds of G-II, G-III and G-IV groups were $3.39 \pm 0.08 \times 10^6/\mu l$, $3.44 \pm 0.06 \times 10^6/\mu l$ and $3.58 \pm 0.04 \times 10^6/\mu l$ respectively. At sixth week of age there was significant influence ($P<0.05$) of ethinylestradiol+levanorgestrel and α-tocopherol supplementation on TEC in birds of G-II and G-III groups respectively when compared to G-I and G-IV groups.

At eighth week of age there was significant difference ($P<0.05$) in the value of TEC between birds of groups G-I (control), G-II and GI-V whereas TEC values were similar in groups G-I and G-III and between G-III and GI-V. The value of G-I (control) was $3.59 \pm 0.02 \times 10^6/\mu l$ where as, birds of G-II, G-III and G-IV groups were $3.46 \pm 0.04 \times 10^6/\mu l$, $3.61 \pm 0.04 \times 10^6/\mu l$ and $3.70 \pm 0.02 \times 10^6/\mu l$ respectively. At eighth week of age there was significant influence ($P<0.05$) of ethinylestradiol+levanorgestrel on the value of TEC in birds of G-II groups when compared to G-I and G-III groups. The value was the highest in G-IV, which showed a significant ($P<0.05$) influence of combined supplementation of ethinylestradiol+levanorgestrel and α-tocopherol when compared to birds of G-I and G-II while it had similar values as of G-III.

There was no significant effect of treatment among the different groups with regards to age of birds.

Table 5a. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on the haematological parameters of broiler chicken from fourth to eighth week of age, mean ± S.E. (n = 8)

	Total RBC count (millions/µl)			Total leucocyte count (thousands/ µl)			Haemoglobin concentration(g%)			Volume of packed red blood cells (%)		
Age (weeks) / Groups	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth
G-I	3.39 ± 0.08^{x}	3.68 ± 0.05^{cy}	3.59 ± 0.02^{by}	22.63 ± 1.58	24.88 ± 0.97^{c}	17.75 ± 0.90^{a}	9.25 ± 0.34^{x}	10.31 ± 0.21^{by}	9.13 ± 0.13^{ax}	27.88 ± 0.52^{a}	28.63 ± 0.46^{c}	27.38 ± 0.37
G-II	3.43 ± 0.08	3.39 ± 0.08^{a}	3.46 ± 0.04^{a}	21.25 ± 2.37	20.50 ± 1.05^{b}	18.63 ± 0.65^{a}	9.13 ± 0.32	8.69 ± 0.25^{a}	8.75 ± 0.19^{a}	30.38 ± 0.46^{by}	27.50 ±0.46^{abx}	26.75 ± 0.45^{x}
G-III	3.53 ± 0.04	3.44 ± 0.06^{ab}	3.61 ± 0.04^{bc}	21.38 ± 1.05^{y}	17.38 ± 0.80^{ax}	21.00 ± 0.89^{by}	8.56 ± 0.35^{x}	8.38 ± 0.13^{ax}	9.75 ± 0.23^{by}	26.88 ± 0.52^{a}	26.50 ± 0.46^{a}	27.38 ± 0.46
G-IV	3.44 ± 0.07^{x}	3.58 ±0.04^{bcy}	3.70 ± 0.02^{cy}	23.13 ± 1.78	20.50 ± 0.57^{b}	21.75 ± 0.67^{b}	9.25 ± 0.27	8.81 ± 0.19^{ax}	10.31 ± 0.16^{cy}	27.50 ± 1.30^{a}	26.75 ± 0.45^{a}	28.25 ± 0.73

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b,c) in columns differ significantly ($P<0.05$).

Mean ± SE (within periods) bearing superscripts (x,y) in rows differ significantly ($P<0.05$).

Table 5b. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on the erythrocyte indices of broiler chicken from fourth to eighth week of age, mean ± S.E. (n = 8)

	MCV (fl)			MCH (pg)			MCHC (g%)		
Age (weeks) / Groups	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth
G-I	82.44 ± 2.54^{ay}	77.32 ± 1.33^{abxy}	76.20 ± 1.00^{x}	27.30 ± 1.03^{y}	27.95 ± 0.30^{by}	25.39 ± 0.29^{ax}	33.18 ± 1.05^{x}	36.04 ± 0.71^{by}	33.39 ± 0.75^{ax}
G-II	92.49 ± 3.86^{by}	81.17 ± 1.56^{cx}	77.90 ± 1.43^{x}	26.59 ± 0.79	25.58 ± 0.41^{a}	25.24 ± 0.37^{a}	30.02 ± 0.91	31.61 ± 0.88^{a}	32.74 ± 0.70^{a}
G-III	76.94 ± 1.78^{a}	77.10 ± 1.63^{ab}	76.12 ± 1.30	26.50 ± 0.52^{y}	24.72 ± 0.57^{ax}	27.54 ± 0.56^{by}	30.93 ± 1.42^{x}	31.65 ± 0.66^{ax}	36.28 ± 1.21^{by}
G-IV	83.79 ± 3.30^{ay}	74.64 ± 1.79^{ax}	76.31 ± 1.84^{x}	28.04 ± 1.19^{y}	24.60 ± 0.48^{ax}	27.86 ± 0.34^{by}	33.29 ± 0.69^{x}	33.00 ± 0.90^{ax}	36.61 ± 0.78^{by}

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b,c) in columns differ significantly ($P<0.05$).

Mean ± SE (within periods) bearing superscripts (x,y) in rows differ significantly ($P<0.05$).

4.3.2 Haemoglobin (Hb) concentration

At fourth week of age, the concentration of Hb did not differ significantly ($P>0.05$) among the various groups (table 5a) and the values ranged from 8.56 ± 0.35 g% in birds of G-III group (supplemented with α-tocopherol) to 9.25 ± 0.27 g% in birds of G-IV group (fed with ethinylestradiol+levanorgestrel and α-tocopherol).

At sixth week of age the birds of groups G-II, G-III and G-IV had significantly ($P<0.05$) lower Hb values (8.69 ± 0.25 g%, 8.38 ± 0.13 g% and 8.81 ± 0.19 g% respectively.) as compared to those of control group G-I (10.31 ± 0.21g%). There was a significant influence of ethinylestradiol+levanorgestrel and α-tocopherol supplementation on Hb value in birds of ethinylestradiol+levanorgestrel alone fed group (G-II), α-tocopherol fed group (G-III) and birds fed with ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) respectively at sixth week of age.

The Hb concentration of birds at eighth week of age showed same pattern as that of sixth week of age except that G-II had almost similar value as that of control. In birds of G-I and G-II the values were 9.13 ± 0.13 g% and 8.75 ± 0.19 g% respectively which were lower than that in G-III and G-IV (9.75 ± 0.23 g% and 10.31 ± 0.16 g% respectively). There was a significant effect of α-tocopherol and combined treatment on the concentration of Hb when compared to the control group (G-I).

The Haemoglobin concentration showed a fluctuating trend with age. There was no significant influence of age among Hb value in birds of control group (G-I). However, the birds fed with ethinylestradiol+levanorgestrel, α-tocopherol and combination of ethinylestradiol+levanorgestrel and α-tocopherols (G-II, G-III and G-IV) showed a reduction in Hb value at sixth week of age. By eighth week of age, birds of G-II group (fed with ethinylestradiol+levanorgestrel) the value of concentration Hb remained as the lower value while those birds of Group III and IV had higher values than G - II as compared to Hb value at sixth week of age.

4.3.3 Volume of packed red blood cells (VPRC)

At fourth week of age there was significant ($P<0.05$) difference in the VPRC value among group G-II and the rest of the groups (table 5a). The value ranged between 26.88 ± 0.52 % in birds of G-III group (α-tocopherol) to 30.38 ± 0.46 % in birds of G-II group fed with ethinylestradiol+levanorgestrel.

At sixth week of age the birds of groups G-III and G-IV showed significantly ($P<0.05$) lower values of VPRC when compared to the control group (G-I) while G-II (27.50 ± 0.46%) had values which were lower than that in control but the difference was not significant. The VPRC was highest in birds of control group G-I (28.63 ± 0.46%) and lowest for α-tocopherol supplemented group (G-III).

At eighth week of age there was no significant difference in VPRC value among various groups. The value ranged between 26.75 ± 0.45 % in birds of G-II group fed with ethinylestradiol+levanorgestrel and 28.25 ± 0.73 % in birds of G-IV group (supplemented with ethinylestradiol+levanorgestrel and α-tocopherol).

There was no significant influence of age on VPRC values in birds of groups G-I, G-III, and G-IV except in G-II where a significant ($P<0.05$) reduction was observed in the values at sixth (27.50 ± 0.46%) and eighth (26.75 ± 0.45%) week of age when compared to fourth week of age. There was significant influence of ethinylestradiol+levanorgestrel in G-II group with age compared to other groups.

4.3.4 Total leucocyte count (TLC)

At fourth week of age, there was no significant variation ($P>0.05$) in the total leucocyte count (TLC) of broiler chicken among control (G-I) and treatment groups (G-II, G-III and G-IV) as given in table 5a. The values ranged from 21.25 ± 2.37 x 10^3 /µl in group G-II and 23.13 ± 1.78 x 10^3/µl in group G-IV.

At sixth week of age the birds of G-III group fed with α-tocopherol had significantly ($P<0.05$) lower TLC value of 17.38 ± 0.80 x 10^3/µl compared to G-I group (control). There was significant ($P<0.05$) variation in TLC values in birds of control (G-I) group (24.88 ± 0.97 x 10^3/µl) and birds supplemented with

ethinylestradiol+levanorgestrel (G-II) and combination of ethinylestradiol +levanorgestrel and α-tocopherol (G-1V) fed groups (20.50 ± 1.05 and 20.50 ± 0.57 x 10^3/µl respectively). Among the treatment groups of birds there was significant difference (P<0.05) in group G-III groups G-II and G-IV. The dietary ethinylestradiol+levanorgestrel and α-tocopherol supplementation significantly lowered the value of TLC in birds of groups G-II, G-III and G-IV.

At eighth week of age the birds in control group (G-I) and ethinylestradiol +levanorgestrel group (G-II) had significantly (P<0.05) lower value of TLC (17.75 ± 0.90 x 10^3/µl and 18.63 ± 0.65 x 10^3/µl respectively) when compared to that of α-tocopherol group (G-III) and combination of ethinylestradiol +levanorgestrel and α-tocopherol fed (G-IV) group (21 ± 0.89 x 10^3/µl and 21.75 ± 0.67 x 10^3/µl respectively) having higher values than the two other groups.

Among the periods, all the groups (G-I to G-IV) had lower TLC value at eighth week of age than that of the fourth week of age. Birds in G-I and G-II had a significantly (P<0.05) lower value at eighth week of age when compared to fourth week. An exceptional aberrant reduction was shown by birds in G-III (birds fed with α-tocopherol) at sixth week of age compared to both fourth and eighth week values.

4.3.5 Erythrocyte indices

4.3.5.1 Mean corpuscular volume (MCV).

At fourth week of age there was significant difference (P<0.05) in the MCV value between group G-II and the rest of the values ranging between 76.94 ± 1.78 fl in birds of G-III (α-tocopherol) group and 92.49 ± 3.86 fl in birds of G-II group fed with ethinylestradiol+levanorgestrel (table 5b).

At sixth week of age the MCV in birds of G-II group was significantly (P<0.05) higher (81.17 ± 1.56 fl) when compared to birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) and other treatment groups. In birds of G-II, G-III, and G-IV treatment groups values ranged between 74.64 ± 1.79 fl in G-IV group to 81.17 ± 1.56 fl in birds of G-II. There was no significant

influence (P>0.05) of dietary α-tocopherol supplementation on MCV value in birds of group G-III and G-IV whereas dietary ethinylestradiol+levanorgestrel supplementation caused significantly (P<0.05) higher MCV value in G- II group.

At eighth week of age there was no significant (P>0.05) variation in MCV between groups and the highest MCV was recorded in birds of G-II group (77.90 ± 1.43 fl) and lowest MCV in birds of G-III group (76.12 ± 1.30 fl).

Among the periods a significantly (P<0.05) lower MCV value was recorded at sixth week of age in birds of G-I (control) and G-II (ethinylestradiol +levanorgestrel). In birds of other treatment groups (G-III and G-IV) there was no significant influence of age on MCV values.

4.3.5.2 Mean corpuscular haemoglobin (MCH)

At fourth week of age there was no significant difference in MCH value among various experimental groups (table 5b). The birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) had the highest value (28.04 ±1.19 pg) and in birds of α-tocopherol supplemented group (G-III) had the lowest value (26.50 ± 0.52 pg).

At sixth weeks of age in the birds of groups G-II, G-III and G-IV had significantly lower MCH value (P<0.05) than that of control group (G-I). The values were the highest in G-I (27.95 ± 0.30 pg) and lowest in G-IV (24.60 ± 0.48 pg).

At eighth week of age there was significant difference in MCH values (P<0.05) among the birds G-I, G-II, (25.39 ± 0.29 and 25.24 ± 0.37 pg) and that of G-III and G-IV (27.54 ± 0.56 and 27.86 ± 0.34 pg). Both G-I and G-II had significantly lower values (P<0.05) than the birds in G-III and G-IV. Dietary supplementation of α-tocopherol and feeding with combination of ethinylestradiol+levanorgestrel and α-tocopherol had significant influence on MCH values in both G-III and G-IV groups.

There was no significant influence of age on MCH value in birds of control group (G-I) and rest of the groups (G-II, G-III and G-IV) vide table 5b.

4.3.5.3 Mean corpuscular haemoglobin concentration (MCHC).

At fourth week of age there was no significant difference ($P<0.05$) in MCHC value of birds of all treatment groups (table 5b). The values ranged from 30.02 ± 0.91 g% in G-II group (ethinylestradiol+levanorgestrel fed) to 33.29 ± 0.69 g% in G-IV group (birds fed with ethinylestradiol+levanorgestrel and α-tocopherol).

At sixth week of age there was significant difference in MCHC value of G-I group ($P<0.05$) and that of rest of the groups. MCHC values were significantly lower in groups G-II, G-III and G-IV (31.61 ± 0.88 g%, 31.65 ± 0.66 g%, 33.00 ± 0.90 g% respectively) when compared to control group G-I (36.04 ± 0.71 g%).

At eighth week of age both G-I and G-II (32.74 ± 0.70 g% and 33.39 ± 0.75 g% respectively) had significantly lower values ($P<0.05$) than the birds in G-III and G-IV groups (36.28 ± 1.21 g% and 36.61 ± 0.78 g% respectively). Dietary supplementation of α-tocopherol (G-III) and combined supplementation of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) had significant influence on MCH values.

Among the periods there was significant influence of age on MCHC value of birds fed with α-tocopherol (G-III) and combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV). Both groups showed an increase in MCHC values at eighth week of age when compared to sixth week. Birds in G-I group had significant increase in sixth week and then returned to the same level of fourth week. Ethinylestradiol+levanorgestrel fed group (G-II) showed no significant change in MCHC values with the advancing age.

4.4 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α -TOCOPHEROL ON BIOCHEMICAL PARAMETERS

4.4.1 Plasma protein profile

4.4.1.1 Total protein

The total plasma protein concentration for the broiler chicken of the four groups at fourth, sixth and eighth week of age are given in table 6.

At fourth week of age there was no significant variation (P>0.05) in the total plasma protein concentration within groups. The birds of G-IV supplemented with combination of ethinylestradiol+levanorgestrel and α-tocopherol had the lowest total plasma protein concentration (4.95 ± 0.06 g/dl) and α-tocopherol supplemented group G-III had the highest total plasma protein value (5.02 ± 0.12 g/dl).

At sixth week of age there was significant difference (P<0.05) in total plasma protein value between birds of G-III, which was lower than the rest of experimental groups. The birds in group G-III had a total plasma protein concentration of 4.92 ± 0.07 g/dl and birds of G-I, G-II and G-IV had total protein values of 5.14 ± 0.09, 5.23 ± 0.05 and 5.15 ± 0.07 g/dl respectively. Alpha-tocopherol failed to show any influence on the plasma total protein concentration.

At eight week age there was no significant difference (P>0.05) in plasma protein concentration of control group G-I with rest of the groups. The α-tocopherol supplemented group G-III had the lowest total plasma protein concentration (4.91 ± 0.08 g/dl) and ethinylestradiol+levanorgestrel supplemented group (G-II) had the highest plasma protein concentration (5.13 ± 0.05 g/dl).

There was no significant influence of age on the total plasma protein concentration at fourth, sixth and eighth week of age in birds of control group G-I and treatment groups G-II, G-III, and G-IV respectively.

4.4.1.2 Albumin

The plasma albumin concentration of broiler chicken at fourth, sixth and eighth week for all four treatment groups are given in table 6. At fourth week of age there was no significant difference in albumin concentration of various treatment groups and lowest value (1.92 ± 0.03 g/dl) was observed in birds of α-tocopherol supplemented group G-III and highest albumin concentration (2.00 ± 0.01 g/dl) was recorded in ethinylestradiol+levanorgestrel fed group G-II.

At sixth week of age, there was significant difference (P<0.05) in plasma albumin concentration in groups G-III and against albumin concentration in group

G-I (control) and group G-II (ethinylestradiol+levanorgestrel). The birds of α-tocopherol supplemented group (G-III) had the lowest albumin concentration of 1.90 ± 0.02 g/dl whereas the birds of group G-II (1.98 ± 0.02 g/dl) had the highest albumin concentration.

At eighth week of age also, there was significant variation ($P<0.05$) in plasma albumin concentration in birds of control group G-I compared to the rest of the groups. The lowest value was observed in birds of control group G-I. There was no significant influence of age on albumin concentration at fourth, sixth and eighth week of age in control group G-I and treatment groups of G-II, G-III and G-IV (table 6).

4.4.1.3 Globulin

The fourth, sixth and eighth week value of plasma globulin concentration for four treatment groups are given in table 6.

At fourth week of age there was no significant variation in plasma globulin concentration among the various groups. The highest plasma globulin value was recorded in birds of α-tocopherol supplemented group G-III (3.13 ± 0.12 g/dl) and lowest globulin concentration was observed in birds of ethinylestradiol+levanorgestrel fed group G-II (2.90 ± 0.15 g/dl).

At sixth week of age there was no significant difference in globulin concentration among the various groups. The birds of α-tocopherol supplemented group G-III had a value of 2.98 ±0.08 g/dl. The birds in groups G-I, G-II and G-IV had comparatively higher globulin values (3.20 ±0.1 g/dl, 3.19 ± 0.07 g/dl and 3.22 ± 0.06 g/dl respectively) than group G-III (fed with α-tocopherol alone).

At eighth week of age too there was no significant variation in plasma globulin values of control group G-I compared to that of groups G-II, G-III and G-IV. The birds of group G-II fed with dietary ethinylestradiol+levanorgestrel supplementation had the highest value (3.18 ± 0.06 g/dl) and α-tocopherol

Table 6. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on plasma protein profile of broiler chicken from fourth to eighth week of age, mean ± S.E. (n = 8)

	Total Protein (g/dl)			Albumin (g/dl)			Globulin (g/dl)			A/G ratio		
Age (weeks) / Groups	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth
G-I	4.99 ± 0.10	5.14 ± 0.09^{b}	5.10 ± 0.06	1.93 ± 0.05	1.96 ± 0.03^{b}	1.87 ± 0.03^{a}	3.01 ± 0.08	3.20 ± 0.10	3.15 ± 0.09	0.64 ± 0.02	0.62 ± 0.03	0.59 ± 0.02
G-II	5.01 ± 0.13	5.23 ± 0.05^{b}	5.13 ± 0.05	2.00 ± 0.01	1.98 ± 0.02^{b}	1.98 ± 0.01^{b}	2.90 ± 0.15	3.19 ± 0.07	3.18 ± 0.06	0.67 ± 0.03	0.62 ± 0.02	0.62 ± 0.02
G-III	5.02 ± 0.12	4.92 ± 0.07^{a}	4.91 ± 0.08	1.92 ± 0.03	1.90 ± 0.02^{a}	1.97 ± 0.02^{b}	3.13 ± 0.12	2.98 ± 0.08	3.01 ± 0.08	0.62 ± 0.03	0.64 ± 0.01	0.65 ± 0.02
G-IV	4.95 ± 0.06	5.15 ± 0.07^{b}	5.10 ± 0.06	1.93 ± 0.04	1.92 ±0.02^{ab}	1.97 ± 0.01^{b}	3.01 ± 0.05	3.22 ± 0.06	3.13 ± 0.05	0.64 ± 0.02	0.59 ± 0.01	0.63 ± 0.01

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b) in columns differ significantly (P<0.05).

supplemented group G-III had the lowest (3.01 ±0.08 g/dl) plasma globulin concentration.

Among the periods, there was no significant influence of age of the birds on the globulin levels of various groups of G-I, G-II, G-III and G-IV.

4.4.1.4 Albumin: Globulin ratio

The Albumin: globulin (A:G) ratio at fourth, sixth and eighth week of age for the four treatment groups are given in table 6.

At fourth, sixth and eighth week of age there was no significant effect of treatments on A:G ratio of broiler chicken. At fourth week of age the lowest A:G ratio of 0.62 ± 0.03 was observed in birds of α-tocopherol supplemented group G-III and the highest ratio of 0.67 ± 0.03 was recorded in birds of ethinylestradiol+levanorgestrel fed group G-II.

At sixth week of age lowest A:G ratio of 0.59 ± 0.01 was recorded in birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) and highest ratio of 0.64 ± 0.01 was observed in birds of α-tocopherol supplemented group (G-III).

At eighth week of age higher A:G ratio was recorded in birds of α-tocopherol supplemented group G-III (0.65 ± 0.02) whereas in birds of control group G-I had the lowest A:G ratio (0.59 ± 0.02).

Among the periods, there was no significant influence of age on A:G ratio of control group G-I and ethinylestradiol+levanorgestrel supplemented group G-II. Both groups showed a reduction in value through the ages. Alpha-tocopherol supplemented group (G-III) showed a trend of increase in value from fourth week through sixth to the eighth week. Ethinylestradiol+levanorgestrel fed birds showed a decrease in A:G ratio from fourth to eighth week. Similar reduction was also noticed in the α-tocopherol fed birds but the values returned to fourth week level by the age of eighth week.

4.4.2 Effect of ethinylestradiol+levanorgestrel and α-tocopherol on plasma lipid profile

4.4.2.1 Total cholesterol

The plasma cholesterol concentration for various experimental groups in the study at fourth, sixth and eighth week of age is given in table 7a and fig. 3. There was no significant variation in plasma cholesterol concentration among various groups at fourth week of age. The birds of control group G-I had the lowest cholesterol concentration of 128.92 ± 3.22 mg/dl and in birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) had the highest value of 136.33 ± 4.45 mg/dl.

At sixth week of age there was significant variation ($P<0.05$) in plasma cholesterol content of the birds of group, G-II supplemented with ethinylestradiol+levanorgestrel (122.09 ± 3.94 mg/dl) with that of birds supplemented with combination of ethinylestradiol+levanorgestrel and α-tocopherol group, G-IV (152.77 ± 12.10 mg/dl). Dietary ethinylestradiol+levanorgestrel supplemented group G-II showed a significantly ($P<0.05$) lower plasma cholesterol concentration (122 ± 3.94 mg/dl) and this decrease in cholesterol concentration was non significant with control group G-I and α-tocopherol fed group G-III when compared to birds of groups G-II and G-IV.

At eighth week of age also significantly ($P<0.05$) the lowest cholesterol concentration was observed in birds of ethinylestradiol+levanorgestrel supplemented group G-II (103.67 ± 6.26 mg/dl) when compared to the rest of the groups G-I, G-III, G-IV (133.57 ± 4.26 mg/dl, 126.07 ± 4.34 mg/dl and 123.74 ± 7.41 mg/dl respectively). There was no significant variation in cholesterol concentration between groups G-I, G-III and G-IV.

Among the periods, there was no significant influence of age on fourth, sixth and eighth week of age on cholesterol concentration in groups G-I, G-III and G-IV and showed tendency of increase in value by sixth week and then a reduction in

Table 7a. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on plasma lipid profile of broiler chicken from fourth to eighth week of age, mean ± S.E. (n = 8)

	Total Cholesterol (mg/dl)			Triglycerides (mg/dl)			Total Lipids (mg/dl)		
Age (weeks) / Group	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth
G-I	128.92 ± 3.22	135.75 ± 4.40^{ab}	133.57 ± 4.26^{b}	108.86 ± 2.74^{a}	109.73 ± 1.77^{b}	108.62 ± 1.46^{c}	529.89 ± 20.02	537.97 ± 19.69^{a}	513.59 ± 14.94^{b}
G-II	131.97 ± 3.53^{y}	122.09 ± 3.94^{ay}	103.67 ± 6.26^{ax}	120.51 ± 2.59^{bz}	60.24 ± 9.99^{ay}	33.71 ± 2.77^{bx}	548.23 ± 10.07^{y}	498.97 ± 31.08^{ay}	409.68 ± 28.54^{ax}
G-III	130.96 ± 2.66	136.88 ± 5.25^{ab}	126.07 ± 4.34^{b}	113.05 ± 1.65^{az}	65.91 ± 6.24^{ay}	24.14 ± 0.90^{ax}	545.18 ± 16.26^{x}	659.81 ± 38.77^{by}	585.58 ± 33.41^{bxy}
G-IV	136.33 ± 4.45	152.77 ± 12.10^{b}	123.74 ± 7.41^{b}	109.07 ± 1.74^{az}	60.91 ± 8.02^{ay}	27.91 ± 2.13^{ax}	552.15 ± 17.98	542.68 ± 26.77^{a}	531.27 ± 21.98^{b}

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b,c) in columns differ significantly ($P<0.05$).

Mean ± SE (within periods) bearing superscripts (x,y,z) in rows differ significantly ($P<0.05$).

Table 7b. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on plasma cholesterol fractions of broiler chicken from fourth to eighth week of age, mean ± S.E. (n = 8)

	HDL (mg/dl)			LDL (mg/dl)			VLDL (mg/dl)		
Age (weeks) / Group	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth
G-I	41.28 ± 2.39^{x}	51.65 ± 2.55^{ay}	41.31 ± 2.37^{ax}	65.87 ± 4.22	62.15 ± 5.67	70.53 ± 4.68^{c}	21.77 ± 0.55^{a}	21.94 ± 0.35^{b}	21.72 ± 0.29^{c}
G-II	41.28 ± 2.41^{x}	77.78 ± 4.86^{by}	71.11 ± 5.90^{by}	66.58 ± 3.89^{y}	32.25 ± 5.21^{x}	25.80 ± 7.15^{ax}	24.10 ± 0.52^{bz}	12.04 ± 2.00^{ay}	6.74 ± 0.55^{bx}
G-III	41.47 ± 2.33^{x}	73.98 ± 4.23^{by}	74.26 ± 4.67^{by}	66.87 ± 3.83^{y}	49.71 ± 6.48^{x}	46.97 ± 2.19^{bx}	22.61 ± 0.33^{az}	13.18 ± 1.25^{ay}	4.82 ± 0.18^{ax}
G-IV	41.15 ± 2.29^{x}	90.92 ± 4.18^{cz}	72.43 ± 2.67^{by}	73.36 ± 5.73^{y}	49.66 ± 13.23	45.73 ± 5.82^{bx}	21.81 ± 0.35^{az}	12.18 ± 1.60^{ay}	5.58 ± 0.43^{ax}

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b,c) in columns differ significantly ($P<0.05$).

Mean ± SE (within periods) bearing superscripts (x,y,z) in rows differ significantly ($P<0.05$).

cholesterol concentration. Whereas, birds in ethinylestradiol+levanorgestrel supplemented group (G-II) had shown significant reduction ($P<0.05$) in cholesterol concentration from fourth to eighth week of age (table 7a).

4.4.2.2 High density lipoprotein (HDL) cholesterol

Effect of ethinylestradiol+levanorgestrel and α-tocopherol supplementation on plasma HDL cholesterol in the four treatment groups at fourth, sixth and eight week are given in table 7b and fig. 4. At fourth week of age there was no significant difference in plasma HDL cholesterol value among the various groups. The HDL cholesterol concentration was lowest (41.15 ± 2.29 mg/dl) in birds of G-IV group (fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol) and the highest value (41.47 ± 2.33 mg/dl) was recorded in birds of G-III group fed with α-tocopherol.

At sixth week the birds of G-IV group (ethinylestradiol+levanorgestrel and α-tocopherol) had significantly ($P<0.05$) higher (90.92 ± 4.18 mg/dl) plasma HDL cholesterol concentration when compared to the ethinylestradiol+levanorgestrel fed group G-II (77.78 ± 4.86 mg/dl) and control group G-I (51.65 ± 2.55 mg/dl), which was the lowest, and α-tocopherol fed group G-III had a value of 73.98 ± 4.23 mg/dl.

At eighth week of age the control group G-I had significantly ($P<0.05$) the lowest (41.31 ± 2.37 mg/dl) HDL cholesterol concentration when compared to that of groups G-II, G-III and G-IV having the value as 71.11 ± 5.90 mg/dl, 74.26 ± 4.67 mg/dl (highest) and 72.43 ± 2.67 mg/dl respectively.

Among the periods, the HDL cholesterol concentration was significantly ($P<0.05$) lower in all experiment groups at the fourth week of age. Broiler chickens of groups G-I, G-II and G-IV recorded significant increase ($P<0.05$) in their HDL cholesterol values by sixth week (highest value). Whereas in α-tocopherol treated group G-III had significant increase (highest value) in eighth week of age. In G-IV group (ethinylestradiol+levanorgestrel and α-tocopherol) there was significant

(P<0.05) decrease in HDL cholesterol concentration at eighth week of age compared to sixth week of age.

4.4.2.3 Low density lipoprotein (LDL) cholesterol

The effect of ethinylestradiol+levanorgestrel and α-tocopherol on plasma concentration of LDL cholesterol at fourth, sixth and eighth week of age for the four treatment groups are given in table 7b and fig. 5. At fourth week of age within groups there was no significant variation in the level of plasma LDL cholesterol. The lowest (65.87 ± 4.22 mg/dl) level of LDL cholesterol was observed in birds of control group G-I and the highest LDL cholesterol concentration (73.36 ± 5.73 mg/dl) was recorded in birds of group G-IV.

At sixth week of age there was significant (P<0.05) lowering in plasma LDL cholesterol value in the ethinylestradiol+levanorgestrel fed group G-II (32.25 ±5.21 mg/dl) when compared to the control group G-I (62.15 ± 5.67 mg/dl). The LDL cholesterol value in other groups did not differ significantly.

The plasma LDL cholesterol values were significantly lower in the ethinylestradiol+levanorgestrel fed group G-II at eighth week of age. It had the lowest value (25.80 ± 7.15 mg/dl) among the various groups. The birds of control group G-I had the highest value of 70.53 ± 4.68 mg/dl. Alpha-tocopherol fed group G-III and the group fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) had significantly (P<0.05) lower value of 46.97 ± 2.19 mg/dl and 45.73 ± 5.82 mg/dl respectively when compared to control group G-I. Both G-III and G-IV also had significantly (P<0.05) higher values when compared to ethinylestradiol+levanorgestrel fed group G-II

Among the periods, the plasma LDL cholesterol value showed significant (P<0.05) association with age in ethinylestradiol+levanorgestrel fed group (G-II), α-tocopherol fed group (G-III) and the group fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV). In these three groups significantly the lowest values (P<0.05) were recorded at the eighth week of age

Fig. 3. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-*a*-tocopherol acetate on the plasma concentration of total cholesterol of broiler chicken from fourth to eighth week of age

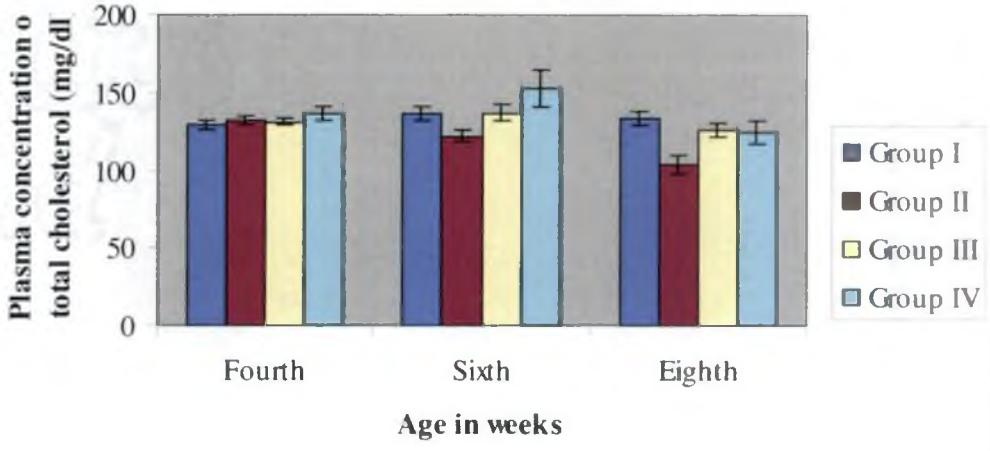
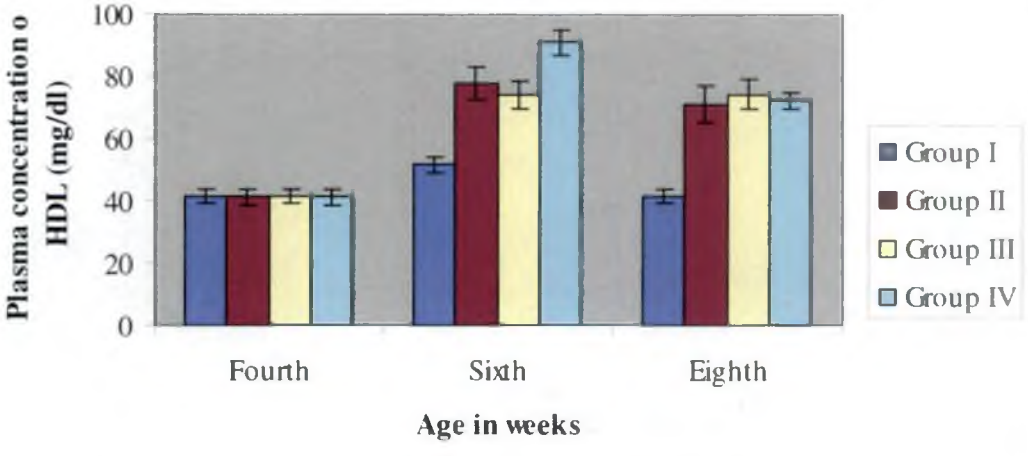


Fig. 4. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-*a*-tocopherol acetate on the plasma concentration of HDL of broiler chicken from fourth to eighth week of age



Group -I- Control ; Group-II- Ethinylestradiol+levanorgestrel fed group; Group-III- dl-α-tocopherol acetate fed group and Group-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

when compared to fourth week. In all the three treatment groups the trend was similar.

4.4.2.4 Very low density lipoprotein (VLDL) cholesterol

Effect of ethinylestradiol+levanorgestrel and α-tocopherol on plasma VLDL cholesterol in the four treatment groups at fourth, sixth and eight week are given in Table 7b and fig. 6. At fourth week of age there was significant difference ($P<0.05$) in plasma VLDL cholesterol value in ethinylestradiol+levanorgestrel fed group G-II than the rest of the groups G-I, G-III and G-IV. VLDL cholesterol concentration was lowest (21.77 ± 0.55 mg/dl) in birds of G-I group (control) and the highest value (24.10 ± 0.52 mg/dl) was recorded birds of G-II group fed with ethinylestradiol+levanorgestrel.

At sixth week of age the birds of control group G-I had significantly ($P<0.05$) the highest (21.94 ± 0.35 mg/dl) plasma VLDL cholesterol concentration when compared to the ethinylestradiol+levanorgestrel fed group G-II (12.04 ± 2 mg/dl), which was the lowest than the α-tocopherol fed group G-III (13.18 ± 1.25 mg/dl) and combination of ethinylestradiol+levanorgestrel and α-tocopherol fed group G-IV (12.18 ± 1.60 mg/dl). All the three treatment groups had significantly lower VLDL cholesterol values than the control group.

At eighth week of age the trend in VLDL cholesterol values were similar to the one found in the sixth week. The exception was only in the lowest value which was observed in the α-tocopherol fed group rather than the Ethinylestradiol+levanorgestrel fed group as was the case in sixth week. There was significant difference ($P<0.05$) in VLDL cholesterol value of the control group, G-I (21.72 ± 0.29 mg/dl) and the rest of the groups G-II, G-III and G-IV (6.74 ± 0.55 mg/dl, 4.82 ± 0.18 mg/dl, 5.58 ± 0.43 mg/dl).Among the periods, the VLDL cholesterol concentration remained same in birds of G-I through out the experiment.

Fig. 5. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-*a*-tocopherol acetate on the plasma concentration of LDL of broiler chicken from fourth to eighth week of age

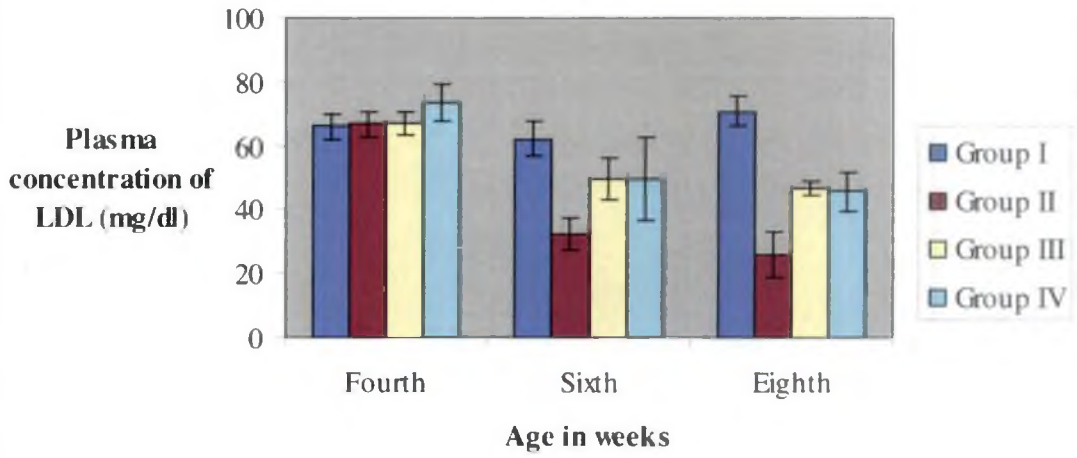
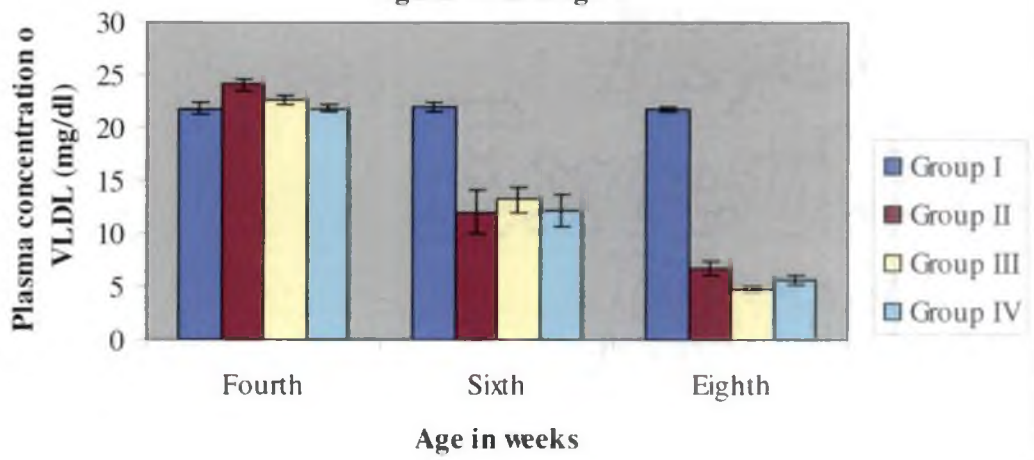


Fig. 6. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-*a*-tocopherol acetate on the plasma concentration of VLDL of broiler chicken from fourth to eighth week of age



Group -I- Control ; Group-II- Ethinylestradiol+levanorgestrel fed group; Group-III- dl-α-tocopherol acetate fed group and Group-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

In all other groups there was a significant reduction of the concentration of VLDL cholesterol from fourth to sixth week of age

and then the trend continued to eighth week of age. In all the three treatments there was drastic reduction in VLDL cholesterol values through out the age.

4.4.2.5 Triglycerides

The effects of ethinylestradiol+levanorgestrel and α-tocopherol on plasma triglycerides concentration in broiler chicken at fourth, sixth and eighth weeks of age are given in table 7a and fig. 7. At fourth week of age there was significant difference ($P<0.05$) in triglycerides concentration among the birds of group G-II than the rest of the groups. The lowest triglycerides concentration (108.86 ± 2.74 mg/dl) was recorded in birds of control group G-I and the highest triglycerides concentration (120.51 ± 2.59 mg/dl) was observed in birds of ethinylestradiol+levanorgestrel fed group G-II.

At sixth week of age, there was significant variation in triglycerides concentration value of control (G-I) and in all the treatment groups (G-II, G-III and G-IV).The control group had the highest (109.73 ± 1.77 mg/dl) triglycerides concentration when compared to that of treatment groups G-II, G-III and G-IV (60.24± 9.99 mg/dl, 65.91 ± 6.24 mg/dl and 60.91 ± 8.02 mg/dl respectively).

At eighth week age significantly ($P<0.05$) the lowest triglycerides value (24.14 ±0.90 mg/dl) was observed in birds of α-tocopherol supplemented group G-III when compared to that of control group G-I (108.62 ± 1.46 mg/dl). There was a significant lowering in triglycerides concentration of ethinylestradiol+levanorgestrel fed group G-II (33.71 ± 2.77 mg/dl) and G-IV fed with ethinylestradiol+levanorgestrel and α-tocopherol (27.91 ± 2.13 mg/dl) when compared to control group G-I.

Among the periods, there was significant influence ($P<0.05$) of age on triglycerides values of treatment groups (G-II, G-III and G-IV) except in the control group G-I. All treatment groups except control group showed a tendency of drastic

Fig. 7. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-*a*-tocopherol acetate on the plasma concentration of triglycerides of broiler chicken from fourth to eighth week of age

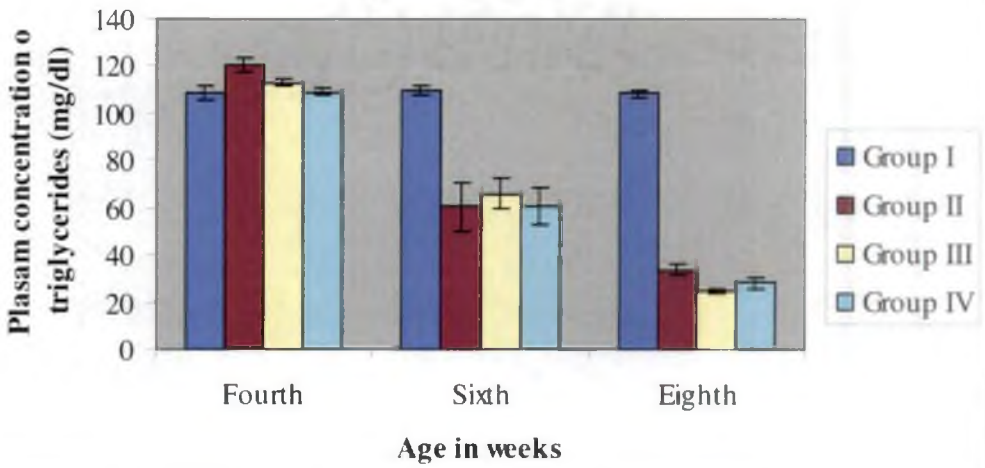
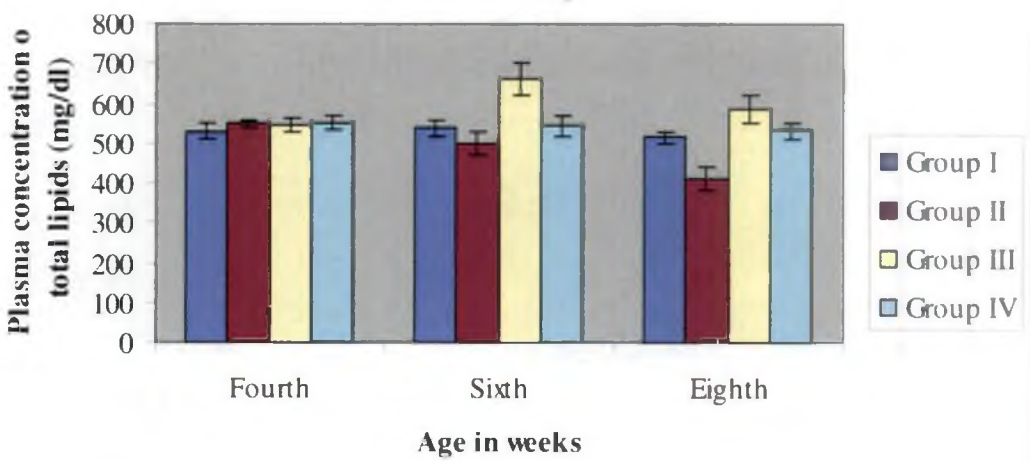


Fig. 8. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-*a*-tocopherol acetate on the plasma concentration of total lipids of broiler chicken from fourth to sixth week of age



Group -I- Control ; Group-II- Ethinylestradiol+levanorgestrel fed group; Group-III- dl-α-tocopherol acetate fed group and Group-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

reduction in triglyceride values with the advancement of age with highest value at fourth week and lowest at eighth week of age.

4.4.2.6 Total lipids

Effect of ethinylestradiol+levanorgestrel and α-tocopherol supplementation on plasma total lipids in the four treatment groups at fourth, sixth and eight weeks are given in Table 7a and fig. 8. At fourth week of age there was no significant difference in plasma total lipids value among the various groups. Total lipids concentration was lowest (529.89 ± 20.02 mg/dl) in birds of G-I group (control) and the highest value (552.15 ± 17.98 mg/dl) was recorded in birds of G-IV group.

At sixth week birds of α-tocopherol fed group G-III had significantly ($P<0.05$) the highest (659.81 ± 38.77 mg/dl) plasma total lipids concentration when compared to the control group G-I (537.97 ±19.69 mg/dl), ethinylestradiol+levanorgestrel fed group G-II had the lowest value of 498.97 ± 31.08 mg/dl while G-IV had the value of 542.68 ± 26.77 mg/dl.

At eighth week of age the ethinylestradiol+levanorgestrel fed group G-II had significantly ($P<0.05$) the lowest (409.68 ± 28.54 mg/dl) total lipid concentration when compared to that of groups G-1, G-III and G-IV (513.59 ± 14.94 mg/dl, 585.58 ± 33.41 mg/dl and 531.27 ± 21.98 mg/dl respectively). The value was highest for birds of G-III group.

Among the periods, the total lipid concentration was significantly ($P<0.05$) higher in birds of α-tocopherol fed group G-III and there was slight increase in the control group G-I at sixth week of age. Both G-I and G-III group recorded reduction in their values by eighth week. Whereas in ethinylestradiol+levanorgestrel treated group there was significant ($P<0.05$) decrease in total lipids with advancing age. The birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol also showed similar trend but was not significant.

4.4.3 Effect of ethinylestradiol+levanorgestrel and α-tocopherol on plasma enzyme profile

4.4.3.1 Gamma glutamyltransferase (GGT)

The plasma concentration of GGT at fourth week of age did not vary significantly among the various groups (table 8 and fig. 9). The birds of group G-IV had the highest value of 19.00 ± 0.80 U/L and in ethinylestradiol+levanorgestrel fed group G-II and α-tocopherol fed group G-III had the lowest value of 17.37 ± 1.29 U/L and 17.37 ± 1.15 U/L respectively.

At sixth week of age there was significant difference ($P<0.05$) in GGT level of ethinylestradiol+levanorgestrel fed group G-II compared to the rest of the groups G-I, G-III and G-IV. The birds of ethinylestradiol+levanorgestrel fed group G-II had GGT concentration of 23.25 ± 0.86 U/L which was the highest and the birds of α-tocopherol supplemented group G-III had the lowest (14.87 ± 1.03 U/L) GGT concentration. Plasma GGT values of both control group G-I (17.50 ± 0.68 U/L) and group G-IV (15.62 ± 1.35 U/L) were significantly lower than the ethinylestradiol+levanorgestrel fed group G-II.

At eighth week of age there was no significant difference ($P>0.05$) in plasma GGT concentration in various experiment groups with the highest value of 21.25 ± 1.70 U/L and lowest value of 16.75 ± 1.24 U/L were observed in birds of ethinylestradiol+levanorgestrel fed group (G-II) and α-tocopherol supplemented group (G-III) respectively.

Among the periods there was significant influence ($P<0.05$) of age in plasma GGT concentration of birds of ethinylestradiol+levanorgestrel fed group (G-II) which showed drastic increase in GGT values with age (highest value at sixth week of age). A non significant reduction in GGT value was observed in α-tocopherol fed group and birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-III and G-IV) at sixth week of age when compared to the values observed at sixth and eighth week of age.

Table 8. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on plasma enzyme profile of broiler chicken from fourth to eighth week of age,
mean ± S.E. (n = 8)

	Gama glutamyltransferase (GGT) (U/L)			Aspartate aminotransferase (AST) (U/L)		
Age (weeks) / Groups	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth
G-I	17.62 ± 0.96	17.50 ± 0.68 ^{a}	17.00 ± 0.96 ^{a}	202.88 ± 4.21	194.63 ± 6.52 ^{a}	210.75 ± 4.51 ^{a}
G-II	17.37 ± 1.29 ^{x}	23.25 ± 0.86 ^{by}	21.25 ± 1.70 ^{bxy}	201.13 ± 8.49 ^{x}	277.38 ± 8.87 ^{by}	263.50 ± 5.80 ^{cy}
G-III	17.37 ± 1.15	14.87 ± 1.03 ^{a}	16.75 ± 1.24 ^{a}	208.75 ± 4.62 ^{x}	214.25 ± 6.56 ^{axy}	231.50 ± 6.34 ^{by}
G-IV	19.00 ± 0.80	15.62 ± 1.35 ^{a}	17.75 ± 0.80 ^{ab}	209.38 ± 6.24 ^{x}	208.25 ± 6.62 ^{ax}	244.88 ± 4.00 ^{by}

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b,c) in columns differ significantly ($P<0.05$).

Mean ± SE (within periods) bearing superscripts (x,y) in rows differ significantly ($P<0.05$).

4.4.3.2 Aspartate aminotransferase (AST)

The plasma AST concentration of broiler chicken at fourth, sixth and eighth week of age for all four experiment groups are given in table 8 and fig. 10.

At fourth week of age there was no significant (P>0 05) difference in plasma AST concentration among various experiment groups. The lowest plasma AST concentration (201.13 ± 8.49 U/L) was observed in birds of G-II group (ethinylestradiol+levanorgestrel fed) and highest plasma AST value (209.38 ± 6.24 U/L) was recorded in birds of G-IV group (fed with a combination of ethinylestradiol+levanorgestrel and α-tocopherol).

At sixth week of age birds of G-II group fed with ethinylestradiol+levanorgestrel had significantly (P<0.05) higher plasma AST concentration (277.38 ± 8.87 U/L) than the rest of the groups and the lowest value was recorded in control group G-I (194.63 ± 6.52 U/L). The birds of α-tocopherol fed group G-III (214.25 ± 6.56 U/L) and birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol, G-IV had significantly (P<0.05) the lower plasma AST concentration (208.25 ± 6.62 U/L) when compared to plasma AST concentration of ethinylestradiol+levanorgestrel fed group G-II.

At eighth week of age significantly (P<0.05) higher plasma AST concentration was observed in birds of ethinyloestrdiol+levanorgestrel fed group G-II (263.50 ± 5.80 U/L). The control group G-I, α-tocopherol fed group G-III and birds fed a combination of ethinylestradiol+levanorgestrel and α-tocopherol G-IV had significantly (P<0.05) lower values (210.75 ± 4.51 U/L, 231.50 ± 6.34 U/L and 244.88 ± 4.00 U/L respectively) when compared to that of ethinylestradiol+levanorgestrel fed group, G-II. Birds fed with α-tocopherol (G-III) and combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) had significantly (P<0.05) higher plasma AST concentration when compared to plasma AST values of control group G-I (210.75 ± 4.51 U/L).

Fig. 9. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on the plasma concentration of GGT of broiler chicken from fourth to eighth week of age

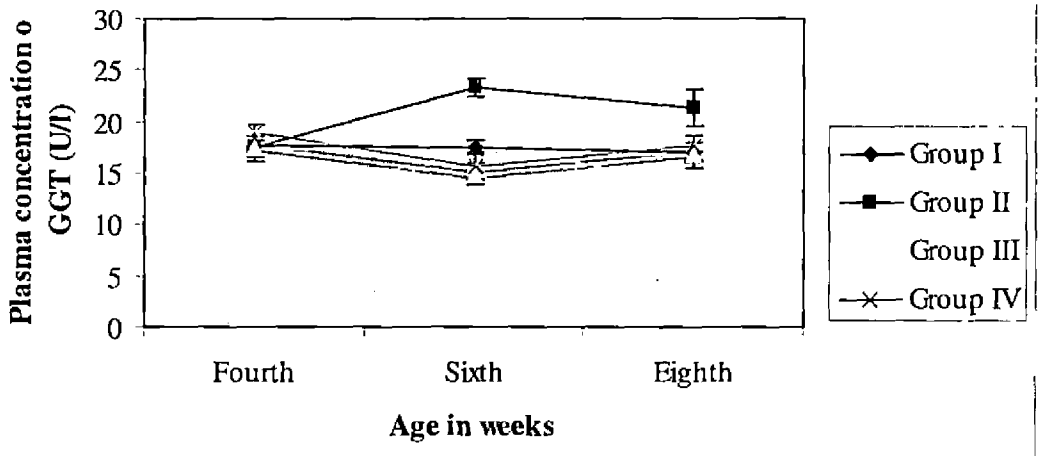
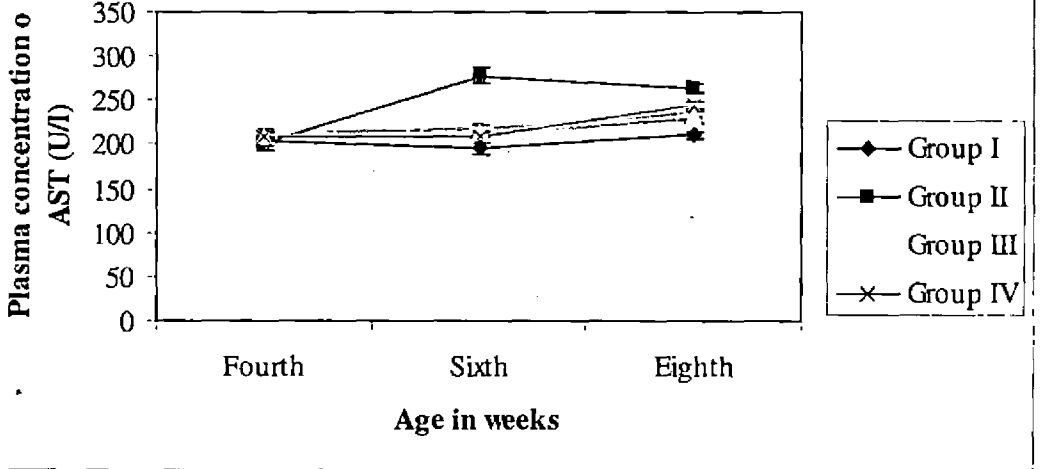


Fig. 10. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on the plasma concentration of AST of broiler chicken from fourth to eighth week of age



Group -I- Control ; Group-II- Ethinylestradiol+levanorgestrel fed group; Group-III- dl-α-tocopherol acetate fed group and Group-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

As the influence of age was compared the values of plasma AST concentration at eighth week of age were found to be significantly ($P<0.05$) higher in all the treatment groups except the control group G-I. Plasma AST values showed significant increase in ethinylestradiol+levanorgestrel fed group (G-II) from fourth to sixth week of age and slight reduction in eight week of age.

4.4.4 Effect of ethinylestradiol+levanorgestrel and α-tocopherol on plasma antioxidant status of broiler chicken.

4.4.4.1 Superoxide desmutase (SOD)

The effect of ethinylestradiol+levanorgestrel and α-tocopherol on plasma concentration of SOD at fourth, sixth and eighth week of age for the four treatment groups are given in table 9 and fig. 11. At fourth week of age within groups there was no significant variation in the level of plasma activity of SOD. The lowest (3676.25 ± 73.12 SOD/ g Hb) level of SOD was observed in birds of α-tocopherol fed group G-III and the highest SOD concentration (4116.38 ± 331.94 SOD/ g Hb) was recorded in birds of control group G-I.

At sixth week of age there was significant ($P<0.05$) variation in plasma SOD value within the groups. The ethinylestradiol+levanorgestrel fed group G-II and α-tocopherol fed group G-III had significantly ($P<0.05$) higher plasma SOD concentration (2542.38 ± 196.78 SOD/ g Hb and 2709.75 ± 125.07 SOD/ g Hb respectively) when compared to that of control group G-I (1837.13 ± 139.07 SOD/ g Hb) in which SOD concentration was the lowest.

The plasma SOD at eighth week of age did not vary significantly among the various groups. The birds of control group G-I had the highest value of 2982.38 ± 183.72 SOD/ g Hb and birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol G-IV had the lowest value of 2764.38 ± 96.37 SOD/ g Hb.

Among the periods, the plasma SOD value was significantly ($P<0.05$) the highest at fourth week of age than that of sixth and eighth week of age in almost all the treatment groups. In control group G-I, there was exceptional reduction in SOD

Table 9. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on plasma antioxidant status of broiler chicken from fourth to eighth week of age, mean ± S.E. (n = 8)

	Superoxide desmutase (SOD/ g Hb)			Catalase (k/ g Hb)			Lipid peroxidation (nmol/ml)		
Age (weeks) / Group	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth	Fourth	Sixth	Eighth
G-I	4116.38 ± 331.94^{z}	1837.13 ± 139.07^{ax}	2982.38 ± 183.72^{y}	2.46 ± 0.17^{x}	4.77 ± 0.45^{az}	3.73 ± 0.34^{y}	2.63 ± 0.11^{y}	1.80 ± 0.14^{bx}	1.81 ± 0.05^{ax}
G-II	3805.63 ± 91.94^{y}	2542.38 ± 196.78^{bc}	2965.38 ± 210.40^{x}	2.49 ± 0.20^{x}	5.22 ± 0.49^{aby}	4.63 ± 0.45^{y}	2.79 ± 0.22^{y}	1.45 ± 0.14^{abx}	2.42 ± 0.22^{by}
G-III	3676.25 ± 73.12^{y}	2709.75 ± 125.07^{cx}	2865.75 ± 113.67^{x}	2.84 ± 0.23^{x}	6.92 ± 0.43^{cz}	5.27 ± 0.82^{y}	2.65 ± 0.11^{y}	1.07 ± 0.06^{ax}	2.25 ± 0.30^{aby}
G-IV	3678.50 ± 69.91^{z}	2086.63 ± 218.22^{ab}	2764.38 ± 96.37^{y}	2.47 ± 0.16^{x}	6.30 ± 0.51^{bcy}	5.56 ± 0.50^{y}	2.52 ± 0.10^{y}	1.36 ± 0.21^{abx}	2.615 ± 0.16^{by}

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b,c) in columns differ significantly ($P<0.05$).

Mean ± SE (within periods) bearing superscripts (x,y,z) in rows differ significantly ($P<0.05$).

value at sixth week and then the value significantly increased at the eighth week of age. In the other groups similar but non significant trend of increase was observed at eighth week when compared to the corresponding value at sixth week of age. The value of SOD was lowest in fourth week of age in all groups.

4.4.4.2 Catalase

The plasma catalase at fourth week of age did not vary significantly among the various groups (table 9 and fig. 12). The birds of α-tocopherol fed group G-III had the highest value of 2.84 ± 0.23 k/ g Hb and in control group G-I had the lowest value of 2.46 ± 0.17 k/ g Hb.

At sixth week of age there was significant difference ($P<0.05$) in catalase level of α-tocopherol fed group G-III compared with groups G-I, and G-II. The birds of α-tocopherol fed group G-III had the highest catalase activity of 6.92 ± 0.43 k/ g Hb. The birds of control group G-I had the lowest (4.77 ± 0.45 k/ g Hb) catalase activity. There was significant difference ($P<0.05$) in catalase activity in birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol fed group, G-IV (6.30 ± 0.51 k/ g Hb) with that of control group G-I. But there was no significant difference between α-tocopherol fed group (G-III) and birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV). Similarly there was no significant difference between ethinylestradiol+levanorgestrel fed group G-II and group G-IV.

At eighth week of age there was no significant difference ($P>0.05$) in plasma catalase activity in various experiment groups with the highest value of 5.56 ± 0.50 k/ g Hb and lowest value of 3.73 ± 0.34 k/ g Hb was observed in birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) and control group G-I respectively. The birds in α-tocopherol fed group G-III (5.27 ± 0.82 k/ g Hb) also had similar values as that in group G-IV.

Fig. 11. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on SOD activity of broiler chicken from fourth to sixth week of age

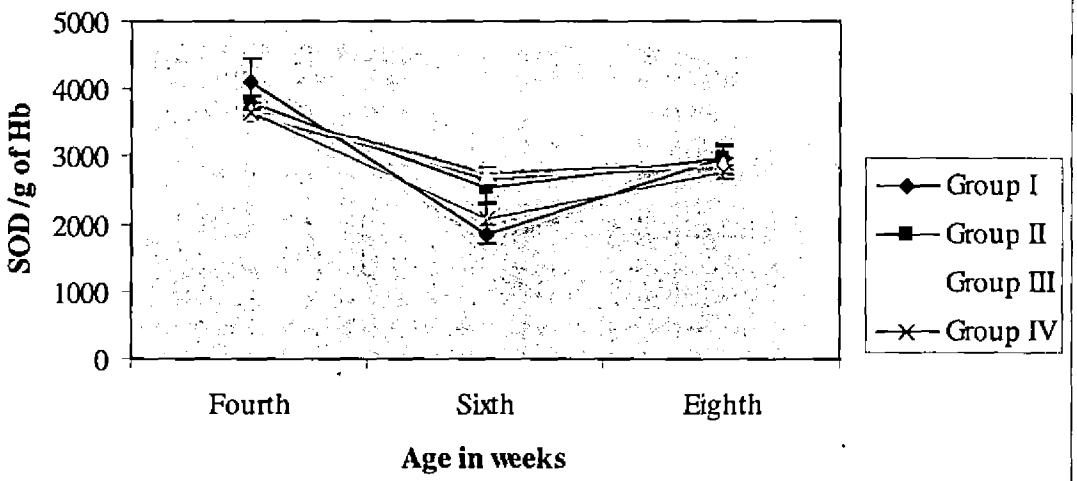
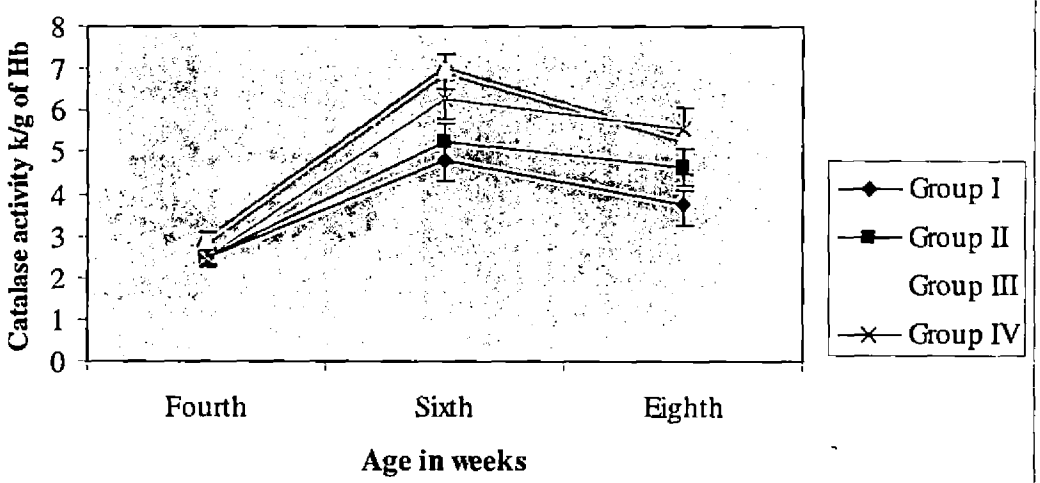


Fig. 12. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on catalase activity of broiler chicken from fourth to eighth week of age



Group -I- Control ; Group-II- Ethinylestradiol+levanorgestrel fed group; Group-III- dl-α-tocopherol acetate fed group and Group-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Among the periods there was significant influence (P>0.05) of age in plasma catalase activity of birds in all the four groups with an increase in activity by the sixth week. The increase was more prominent in birds of α-tocopherol fed group and ethinylestradiol+levanorgestrel and α-tocopherol fed group. The entire groups showed a marginal reduction in catalase activity by the eighth week of age. The change in catalase activity with age was lesser in control group (G-I) when compared with the rest of the groups.

4.4.4.3 Lipid peroxidation

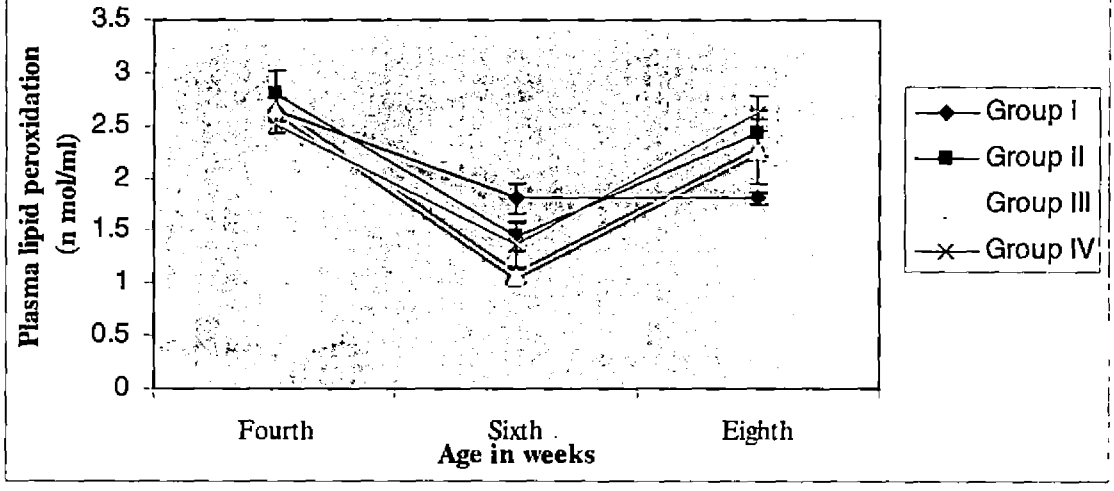
The plasma lipid peroxidation value of broiler chicken at fourth, sixth and eighth week of age for all four-experiment groups are given in table 9 and fig. 13.

At fourth week of age there was no significant (P>0 05) difference in plasma lipid peroxidation concentration among various experiment groups. The lowest plasma lipid peroxidation concentration (2.52 ± 0.10 nmol/ml) was observed in birds of G-IV group fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol and highest plasma lipid peroxidation value (2.79 ± 0.22 nmol/ml) was recorded in birds of G-II group, ethinylestradiol+levanorgestrel fed group.

At sixth week of age birds of G-III group with dietary supplementation of α-tocopherol had significantly (P<0.05) the lowest plasma lipid peroxidation concentration (1.07 ± 0.06 nmol/ml) than the control group G-I (1.80 ± 0.14 nmol/ml). The birds of ethinylestradiol+levanorgestrel fed group G-II (1.45 ± 0.14 nmol/ml) and the birds fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol fed group, G-IV (1.36 ± 0.21 nmol/ml) also had lower values when compared to control group, G-I.

At eighth week of age within groups there was no significant variation in the level of plasma lipid peroxidation. The lowest (1.81 ± 0.05 nmol/ml) level of lipid peroxidation was observed in birds of control group G-I and the highest lipid peroxidation (2.62 ± 0.16 nmol/ml) was recorded in birds of group G-IV fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol.

Fig. 13. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-*a*-tocopherol acetate on plasma lipid peroxidation level of broiler chicken from fourth to eighth week of age



Group -I- Control ; Group-II- Ethinylestradiol+levanorgestrel fed group; Group-III- dl-α-tocopherol acetate fed group and Group-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

As the influence of age was compared, the values of plasma lipid peroxidation at fourth week were found to be significantly ($P<0.05$) higher in all the experiment groups when compared with the values at sixth week of age. In other words all treatment groups recorded lower values at sixth week, with group G-III recording the largest fall in lipid peroxidation when compared to fourth week values. As the birds grew older from sixth to eighth week there was significant increase in lipid peroxidation in all the groups except the control group in which the values remained almost same as the one at sixth week of age.

4.4.5 Effect of ethinylestradiol+levanorgestrel and α-tocopherol on serum minerals

The results of treatment of ethinylestradiol+levanorgestrel and α-tocopherol on serum minerals are given in table 10. At fourth week of age there was no significant variation in the serum concentration of calcium, phosphorous copper and iron among the various groups. The highest calcium, copper, and iron concentrations were recorded in birds of ethinylestradiol+levanorgestrel fed group G-II (Ca - 13.11 ± 1.25 mg %, Cu - 0.24 ± 0.03 ppm and Fe - 3.25 ± 0.20 ppm) and control group G-I had the highest phosphorous concentration (P - 4.59 ± 1.16 mg %). The lowest concentration of calcium, copper and iron were observed in birds of control group G-I (Ca - 11.65 ± 0.05mg %, Cu - 0.19 ± 0.03ppm and Fe - 2.41 ± 0.31ppm) and that for phosphorous was observed in birds of group G-IV group fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (3.49 ± 1.11 mg %).

At sixth week of age there was no significant difference in calcium, copper and iron concentration among the various groups. There was a significant difference ($P<0.05$) in phosphorous concentration among the groups with highest value in ethinylestradiol+levanorgestrel fed group, G-II (6.59 ± 1.16 mg %) and lowest value in birds of α-tocopherol supplemented group, G-III (3.49 ± 1.11 mg %).

Table 10. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on serum concentration of calcium, phospherous, copper and iron in broiler chicken from fourth to eighth week of age, Mean ± S.E. (n = 8)

Group	Age in weeks											
	Fourth				Sixth				Eighth			
	Ca (mg %)	P (mg %)	Cu (ppm)	Fe (ppm)	Ca (mg %)	P (mg %)	Cu (ppm)	Fe (ppm)	Ca (mg %)	P (mg %)	Cu (ppm)	Fe (ppm)
Group I	11.65 ± 0.05	4.59 ± 1.16^{x}	0.19 ± 0.03^{x}	2.41 ± 0.31	11.89 ± 0.11	4.48 ± 0.97^{bx}	0.21 ± 0.01^{x}	3.25 ± 0.20	11.83 ± 0.05	8.64 ± 0.27^{y}	0.13 ± 0.01^{y}	2.04 ± 0.26
Group II	13.11 ± 1.25	4.48 ± 0.97^{x}	0.24 ± 0.03^{x}	3.25 ± 0.20	13.11 ± 2.49	6.59 ± 1.16^{cy}	0.19 ± 0.02^{x}	2.41 ± 0.31	11.65 ± 0.05	7.68 ± 0.60^{z}	0.14 ± 0.01^{y}	1.96 ± 0.35
Group III	11.83 ± 0.05	4.34 ± 0.67	0.19 ± 0.02^{x}	2.81 ± 0.18	11.88 ± 0.46	3.49 ± 1.11^{a}	0.24 ± 0.03^{x}	2.95 ± 0.34	11.93 ± 0.07	8.94 ± 0.58	0.14 ± 0.01^{y}	2.03 ± 0.56
Group IV	11.93 ± 0.07	3.49 ± 1.11	0.21± 0.01^{x}	2.95 ± 0.34	11.16 ± 0.46	4.34 ± 0.67^{b}	0.19 ± 0.03^{x}	2.81 ± 0.18	13.11 ± 1.25	8.11 ± 0.84	0.13 ± 0.02^{y}	2.21 ± 0.41

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group; G-III- dl-α-tocopherol acetate fed group and G-IV- Ethinylestradiol+levanorgestrel+ dl-α-tocopherol acetate fed group.

Mean ± SE (between groups) bearing superscripts (a,b) in columns differ significantly ($P<0.05$).

Mean ± SE (within a group) bearing superscripts (x,y,z) in rows differ significantly ($P<0.05$).

At eighth week of age too there was no significant variation in the concentration of serum calcium, phosphorous, copper and iron values of control group G-I with that of groups G-II, G-III and G-IV.

When the influence of treatment on age was compared there was a significant in crease in phosphorous value by the eighth week in all the four groups. The increase was significant in ethinylestradiol+levanorgestrel fed group at sixth week it self while, in the rest of the groups the increase occurred only at the eighth week. In all the treatment groups copper concentration was significantly lower at the eighth week of age.

4.5 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON TISSUE ENZYME AND ANTIOXIDANT STATUS

4.5.1 Gamma glutamyltransferase (GGT) level in liver and breast muscle

There was significant difference ($P<0.05$) in liver tissue GGT level of ethinylestradiol+levanorgestrel fed group G-II compared with rest of the groups G-I, G-III and G-IV (table 11). The birds of ethinylestradiol+levanorgestrel fed group, G-II had the GGT value of 19 ± 0.80 U/mg of protein which was the highest and the birds of control group, G-I had the lowest (17 ± 0.96 U/mg of protein) GGT concentration. Plasma GGT values of both α-tocopherol fed group, G-III (17.37 ± 1.29 U/mg of protein) and birds of G-IV fed with a combination of ethinylestradiol +levanorgestrel and α-tocopherol (17.75 ± 0.80 U/mg of protein) were significantly lower than the ethinylestradiol+levanorgestrel fed group, G-II. The values in breast muscle did not vary significantly with values ranging from 2.61 ± 0.08 U/mg of protein in birds fed with a combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) to 2.65 ± 0.11 U/mg of protein in ethinylestradiol+levanorgestrel fed group (G-II).

4.5.2 Tissue antioxidant status

4.5.2.1 Peroxidation level in liver and breast muscle.

At slaughter birds of G-III group with dietary supplementation of α-tocopherol had significantly ($P<0.05$) lower tissue lipid peroxidation level (table 11)

Table 11. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on Gama glutamyltransferase activity and peroxidation level in liver and breast muscles of broiler chicken, mean ± S.E. (n = 8)

	GGT (U/mg of protein)		Peroxidation (n mol/ml of homogenate)	
Tissue / Groups	Liver	Breast muscle	Liver	Breast muscle
G-I	17.00 ± 0.96 [b]	2.63 ± 0.11	2.63 ± 0.11 [b]	1.81 ± 0.05
G-II	19.00 ± 0.80 [a]	2.65 ± 0.11	2.65 ± 0.11 [b]	1.80 ± 0.14
G-III	17.37 ± 1.29 [b]	2.61 ± 0.16	1.80 ± 0.14 [a]	1.36 ± 0.21
G-IV	17.75 ± 0.80 [b]	2.61 ± 0.08	2.42 ± 0.22 [b]	1.45 ± 0.14

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group;
G-III- dl-α-tocopherol acetate fed group and G-IV- ethinylestradiol+levanorgestrel + dl-α-tocopherol acetate fed group.
Mean ± SE (between groups) bearing superscripts (a,b) in columns differ significantly (P<0.05).

Table 12. Effect of dietary supplementation of ethinylestradiol+levanorgestrel and dl-α-tocopherol acetate on the level of crude protein and ether extract of broiler chicken, mean ± S.E. (n = 8)

Groups	Crude protein (%)	Ether extract (%)
G-I	19.67 ± 0.21	7.34 ± 0.13 [b]
G-II	19.69 ± 0.10	6.82 ± 0.12 [a]
G-III	19.70 ± 0.13	6.83 ± 0.14 [a]
G-IV	19.67 ± 0.16	6.85 ± 0.13 [a]

G-I- Control group; G-II- Ethinylestradiol+levanorgestrel fed group;
G-III- dl-α-tocopherol acetate fed group and G-IV- ethinylestradiol+levanorgestrel + dl-α-tocopherol acetate fed group.
Mean ± SE (between groups) bearing superscripts (a,b) in columns differ significantly (P<0.05).

in liver and breast muscle (1.80 ± 0.14 and 1.36 ± 0.21 nmol/ml of homogenate respectively) than the control group G-I (2.63 ± 0.11 and 1.81 ± 0.05 nmol/ml of homogenate, liver and breast muscle respectively). The birds of G-IV fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (2.42 ± 0.22 and 1.45 ± 0.14 nmol/ml of homogenate respectively) also had lower values in liver and breast muscle when compared to the control group G-I.

4.6 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON THE LEVEL OF CRUDE PROTEIN AND ETHER EXTRACT OF BREAST MUSCLES.

There was no significant variation in crude protein content among the various groups (table 12). The highest value of crude protein was recorded in birds of α-tocopherol group G-III (19.70 ± 0.13 %) and lowest value was observed in birds of G-IV fed with combination of ethinylestradiol+levanorgestrel and α-tocopherol (19.67 ± 0.16 %). With regard to ether extract, the birds in group G-II, G-III and G-IV (6.82 ± 0.12 %, 6.83 ± 0.14 %, 6.85 ± 0.13 % respectively) had significantly lower ether extract values when compared to control group G-I (7.34 ± 0.13 %).

4.7 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON HISTOMORPHOLOGY OF LIVER, ADRENAL AND SKELETAL MUSCLES.

Morphologically liver from ethinylestradiol+levanorgestrel exposed birds were nearly normal in size, colour and texture. Livers from birds in rest of the groups were larger, fragile and fatty in texture (plate 4 to 8). Tissue sections (plate 1 to 4) also had similar trend with fatty degeneration of liver as the most prevalent condition in birds other than those in the ethinylestradiol+levanorgestrel supplemented group (G – II). Sections of adrenals (plate 9 to 12) from all the four treatment groups were found normal. Skeletal muscle sections (plate 13 to 16) were normal in all the groups of birds.

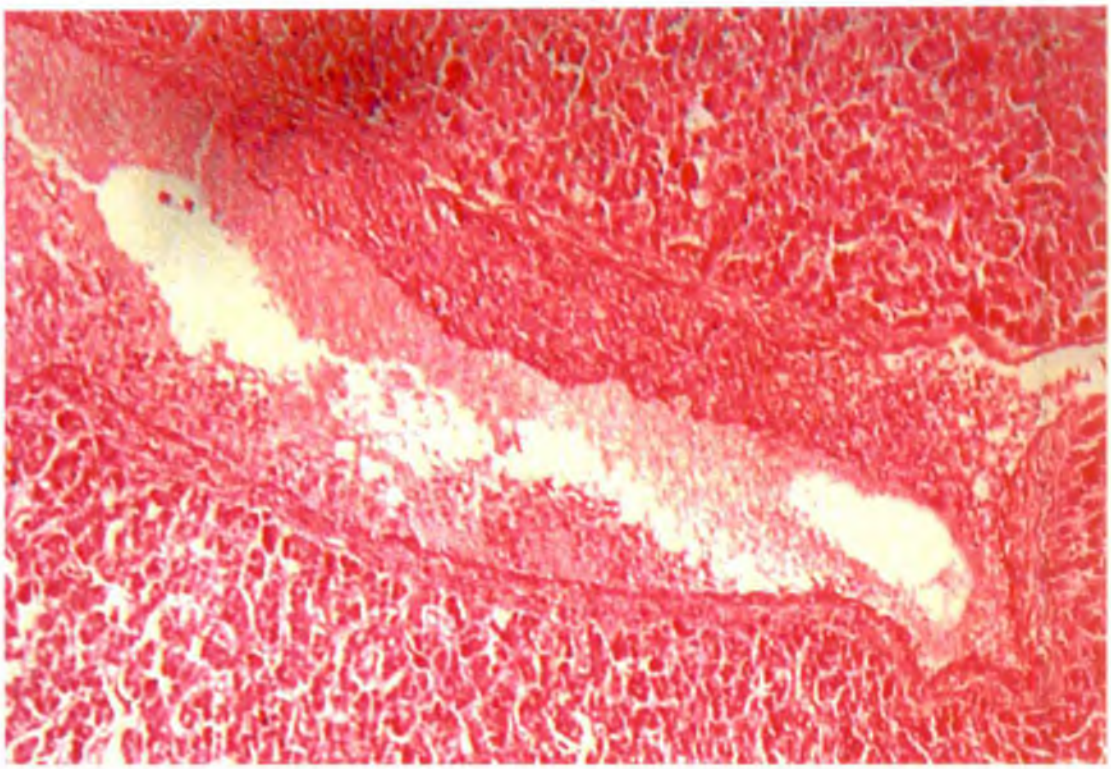


Plate 1. Cross section of liver of control group (G-I)

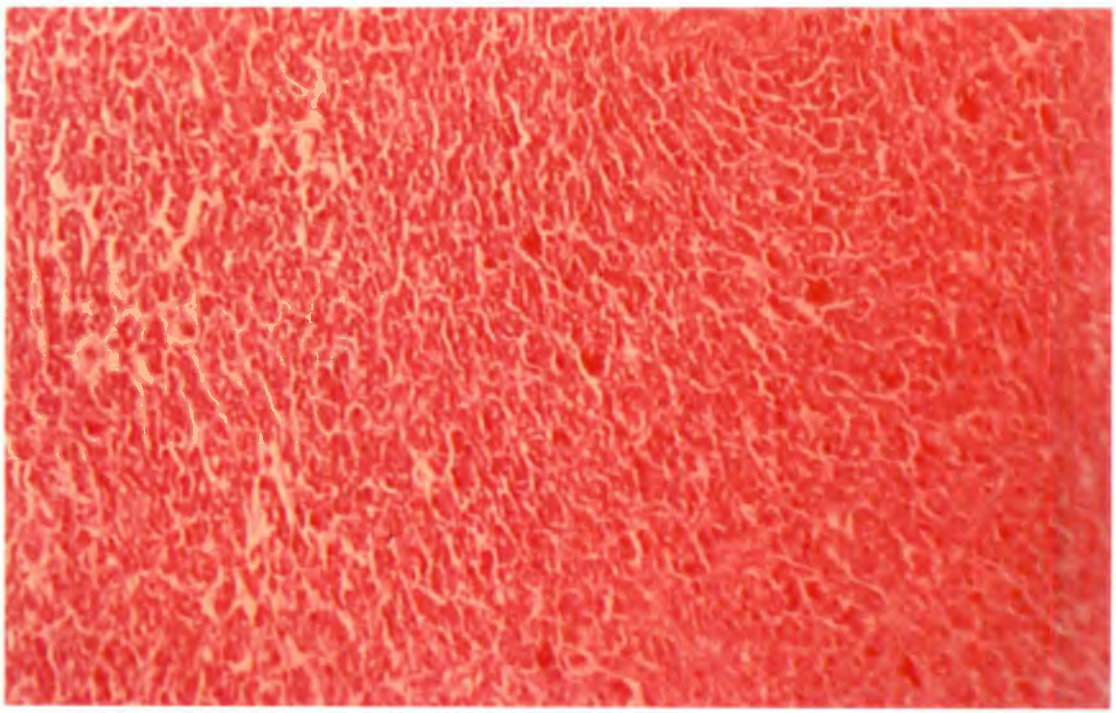


Plate 2. Cross section of liver of ethinylestradiol+levanorgestrel fed group (G-II)

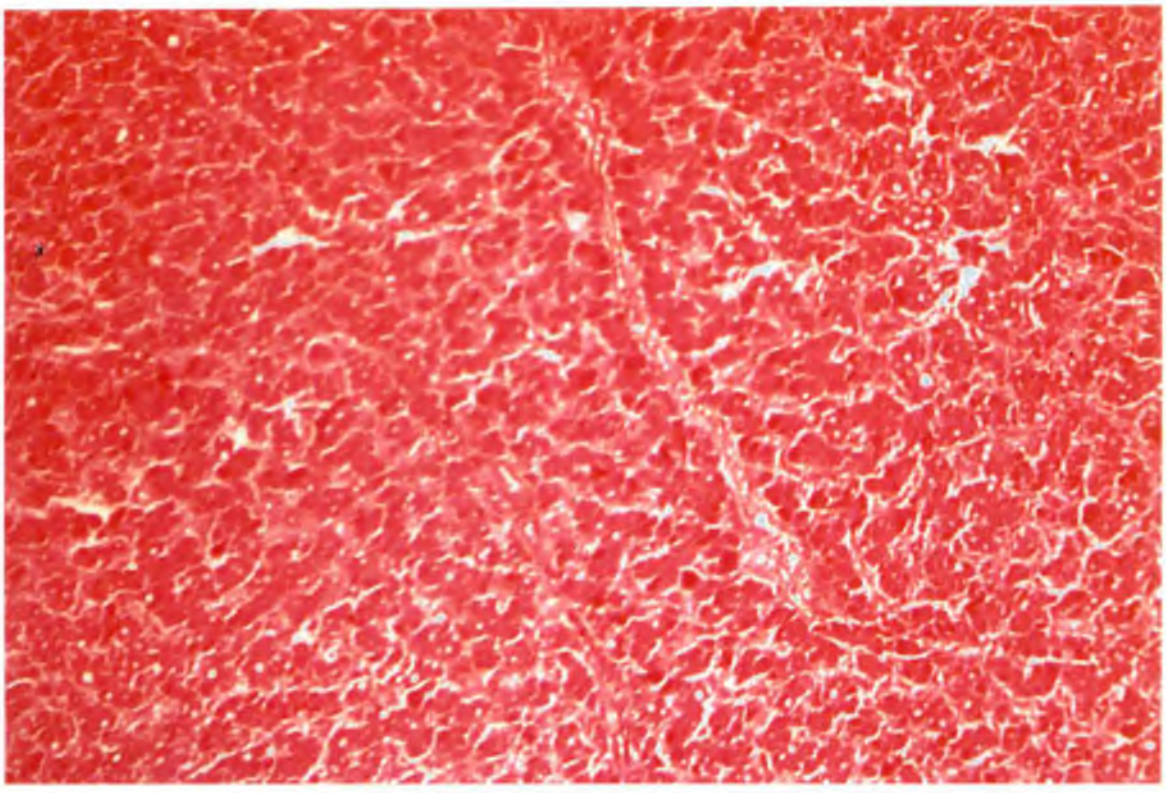


Plate 3. Cross section of liver of alpha-tocopherol fed group (G-III)

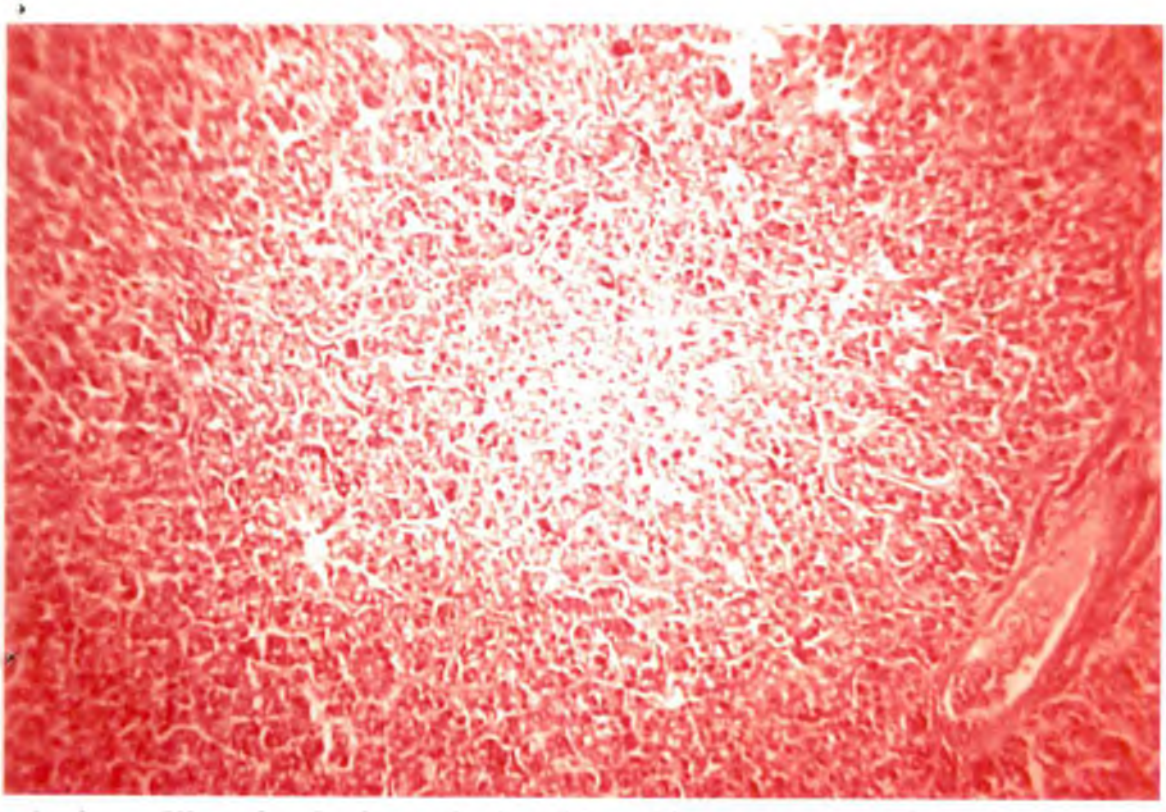


Plate 4. Cross section of liver of ethinylestradiol+levanorgestrel and alpha-tocopherol fed group (G-IV)

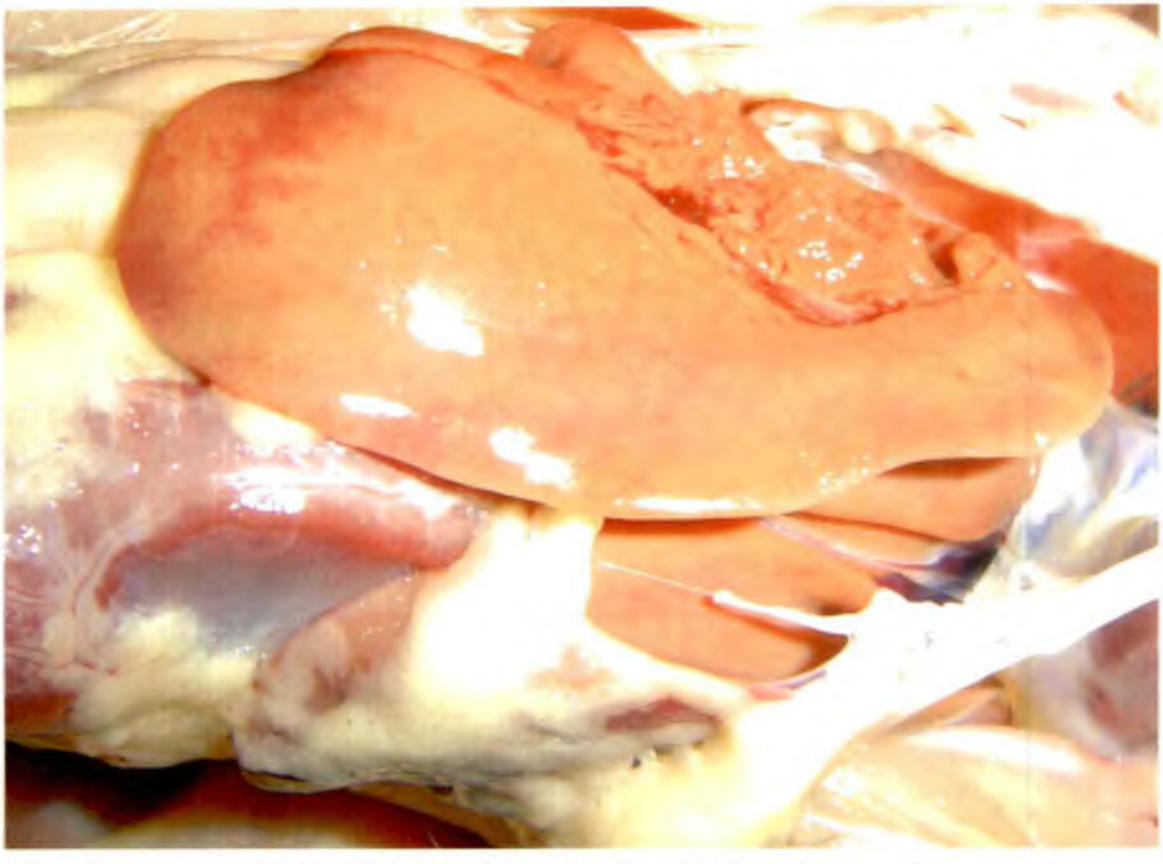


Plate 5. Gross structure of liver of control group (G-I)

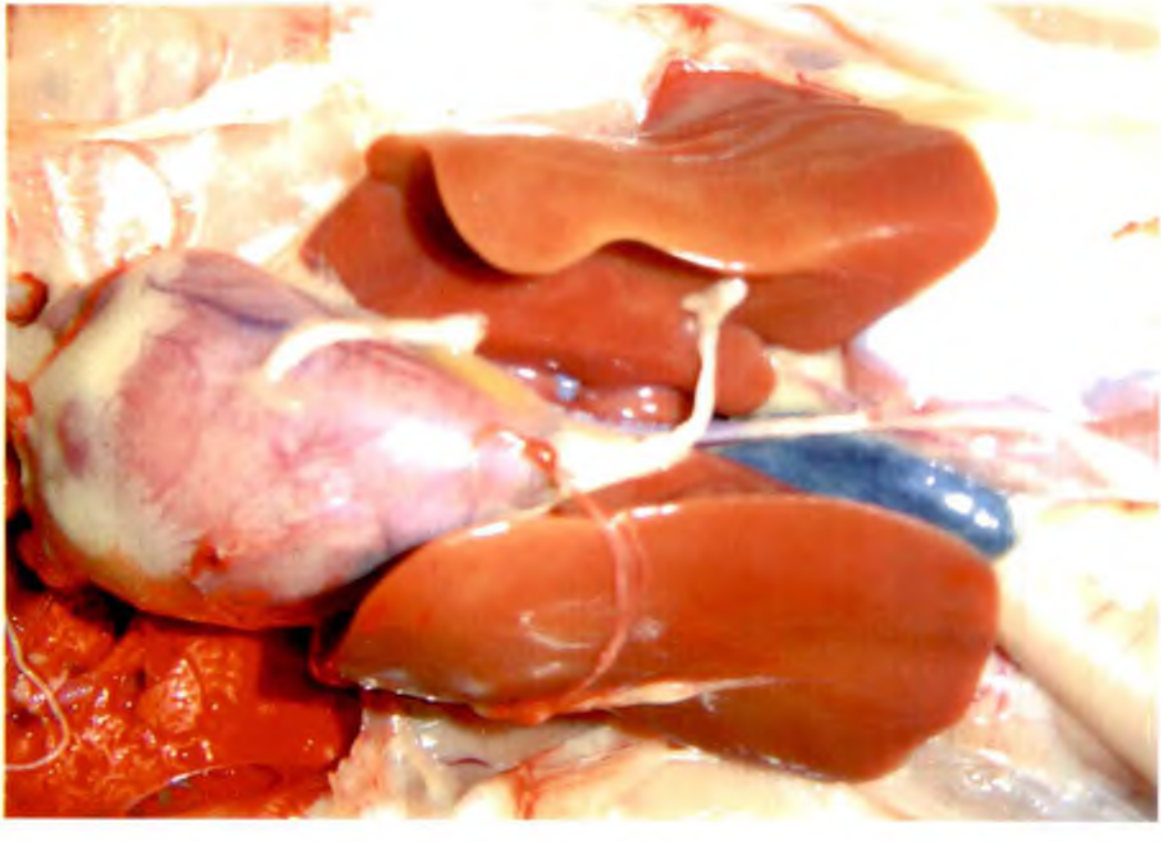


Plate 6. Gross structure of liver of ethinylestradiol+levanorgestrel fed group (G-II)

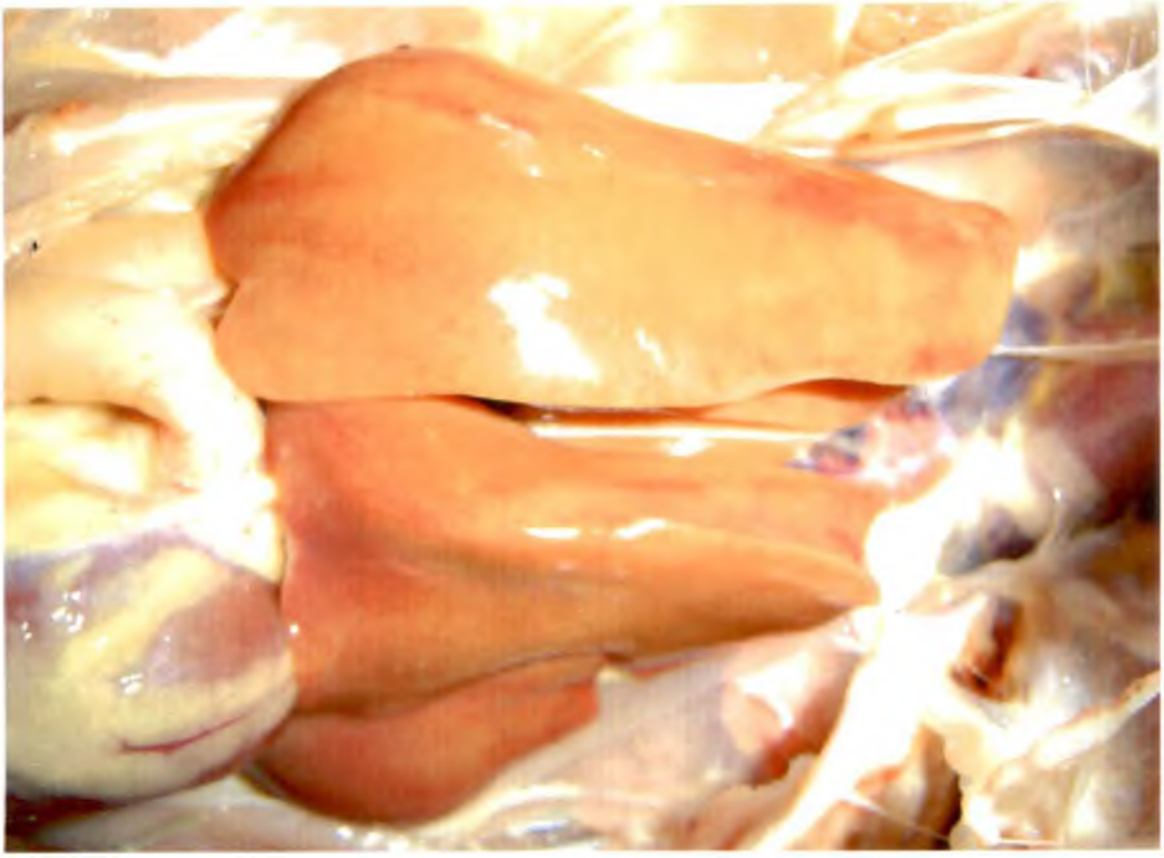


Plate 7. Gross structure of liver of alpha-tocopherol fed group (G-III)

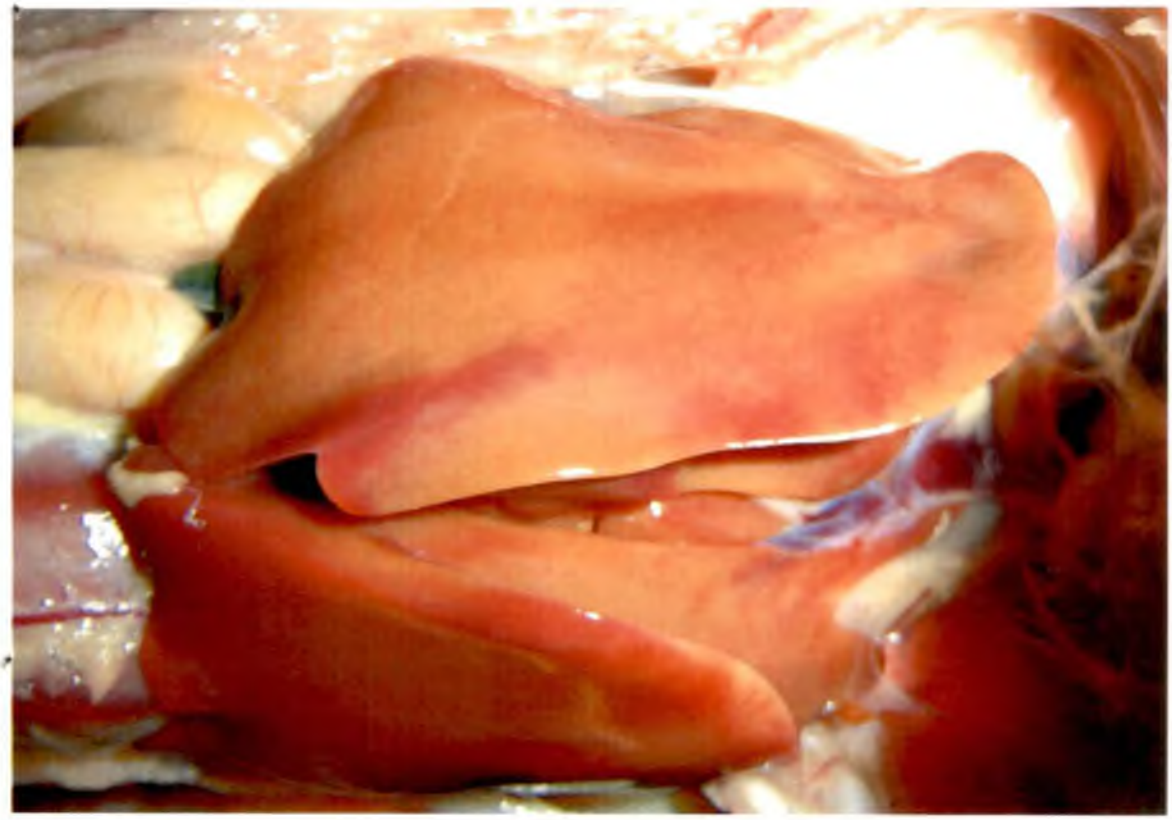


Plate 8. Gross structure of liver of ethinylestradiol+levanorgestrel and alpha-tocopherol fed group (G-IV)

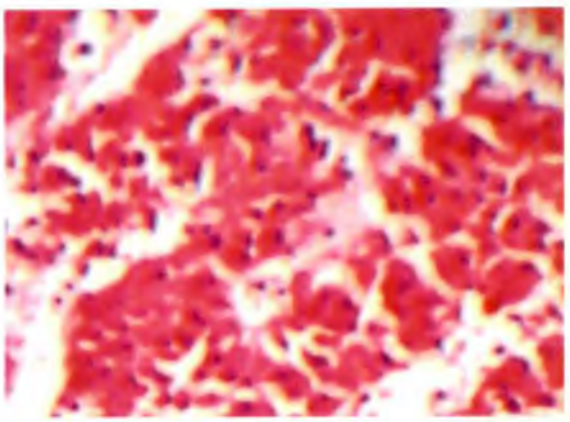


Plate 9. Cross section of adrenal of control group (G-I)

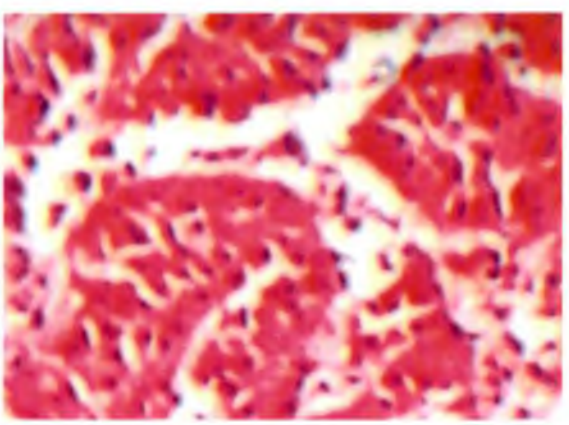


Plate 10. Cross section of adrenal of ethinylestradiol+levanorgestrel fed group (G-II)

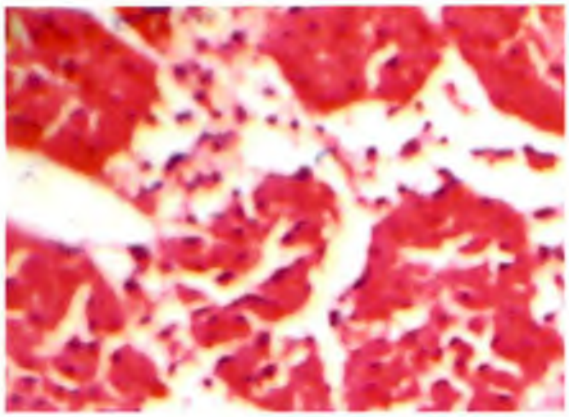


Plate 11. Cross section of adrenal of alpha-tocopherol fed group (G-III)

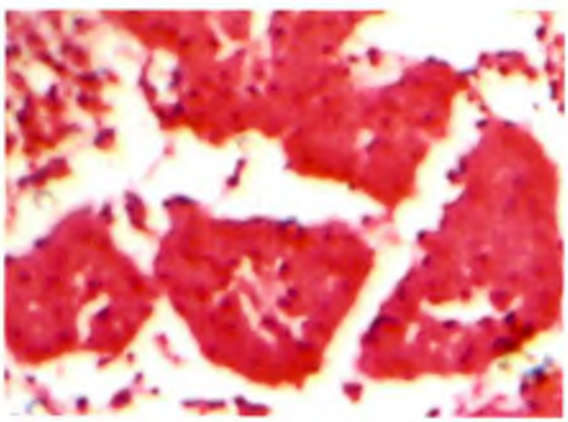


Plate 12. Cross section of adrenal of ethinylestradiol+levanorgestrel and alpha-tocopherol fed group (G-IV)

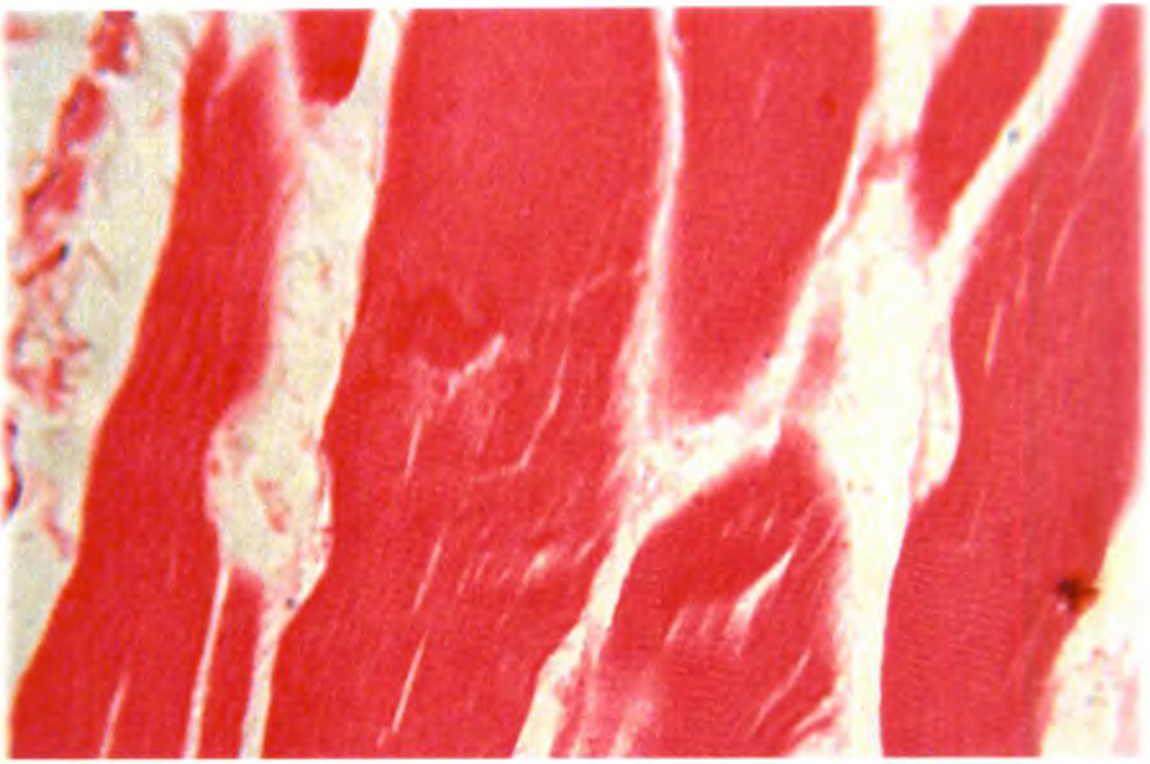


Plate 13.. Longitudinal section of breast muscle of control group (G-I)

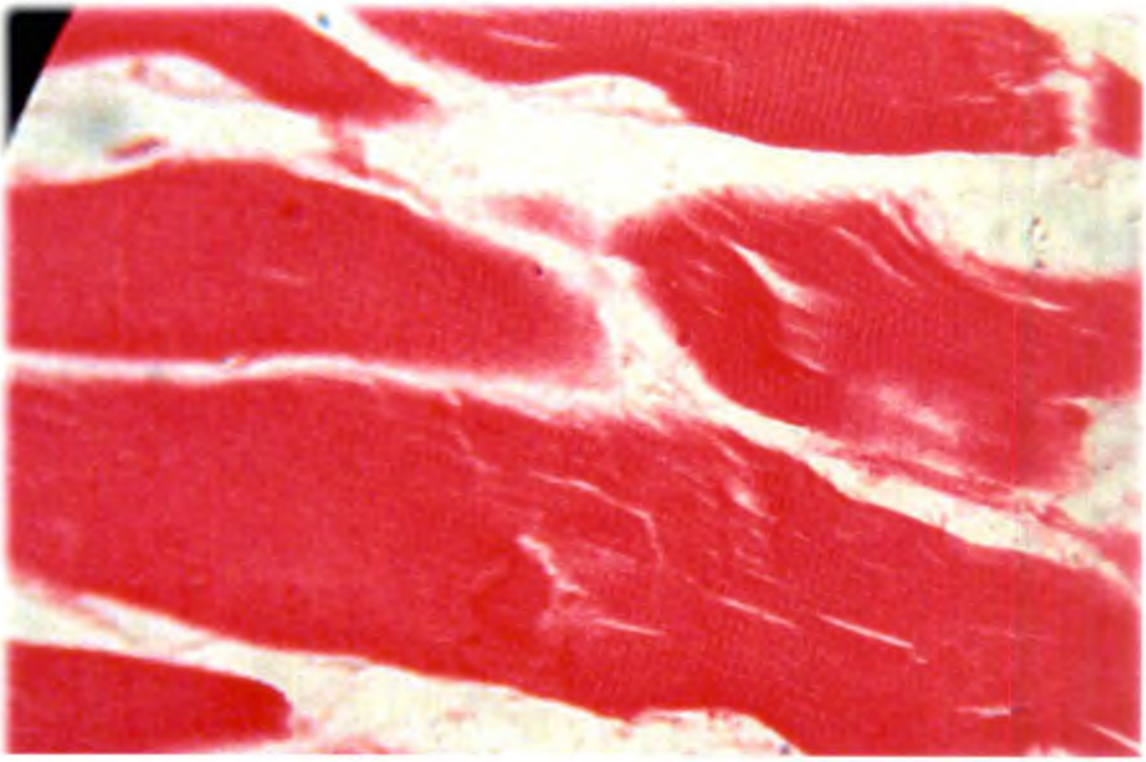


Plate 14. Longitudinal section of breast muscle of ethinylestradiol +levanorgestrel fed group (G-II)

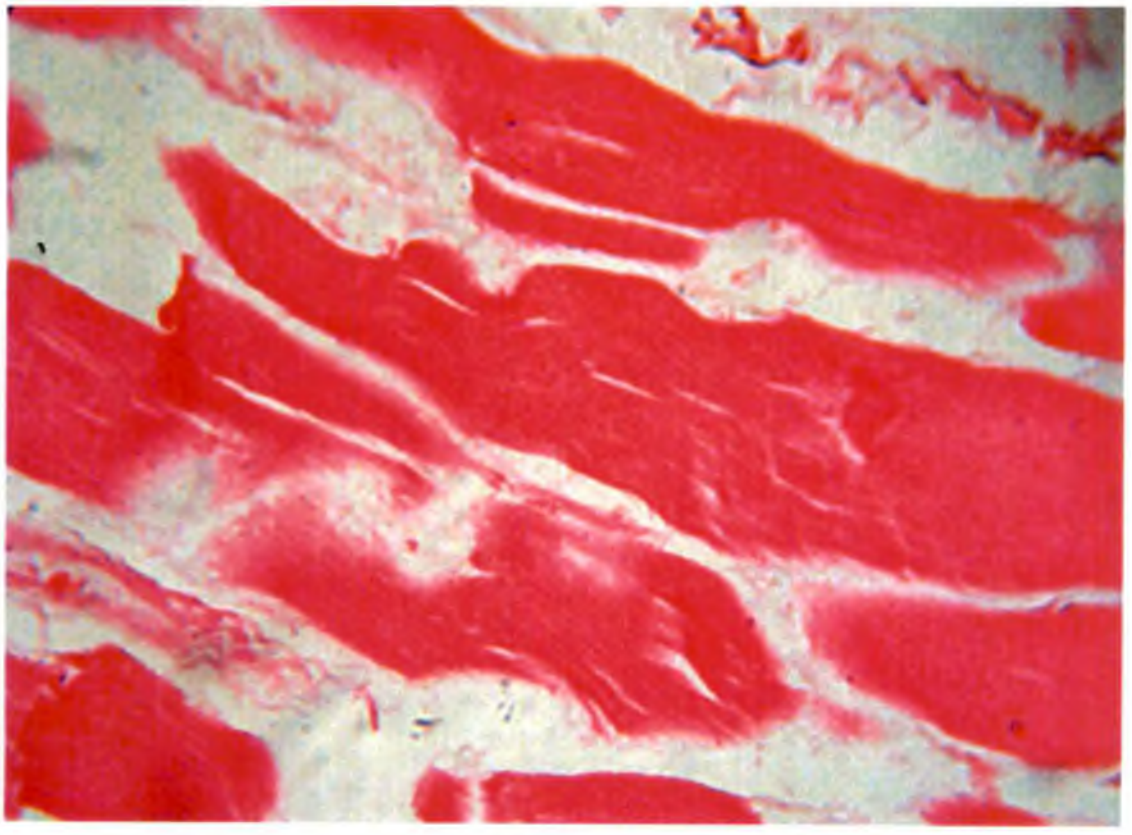


Plate 15. Longitudinal section of breast muscle of alpha-tocopherol fed (group G-III)

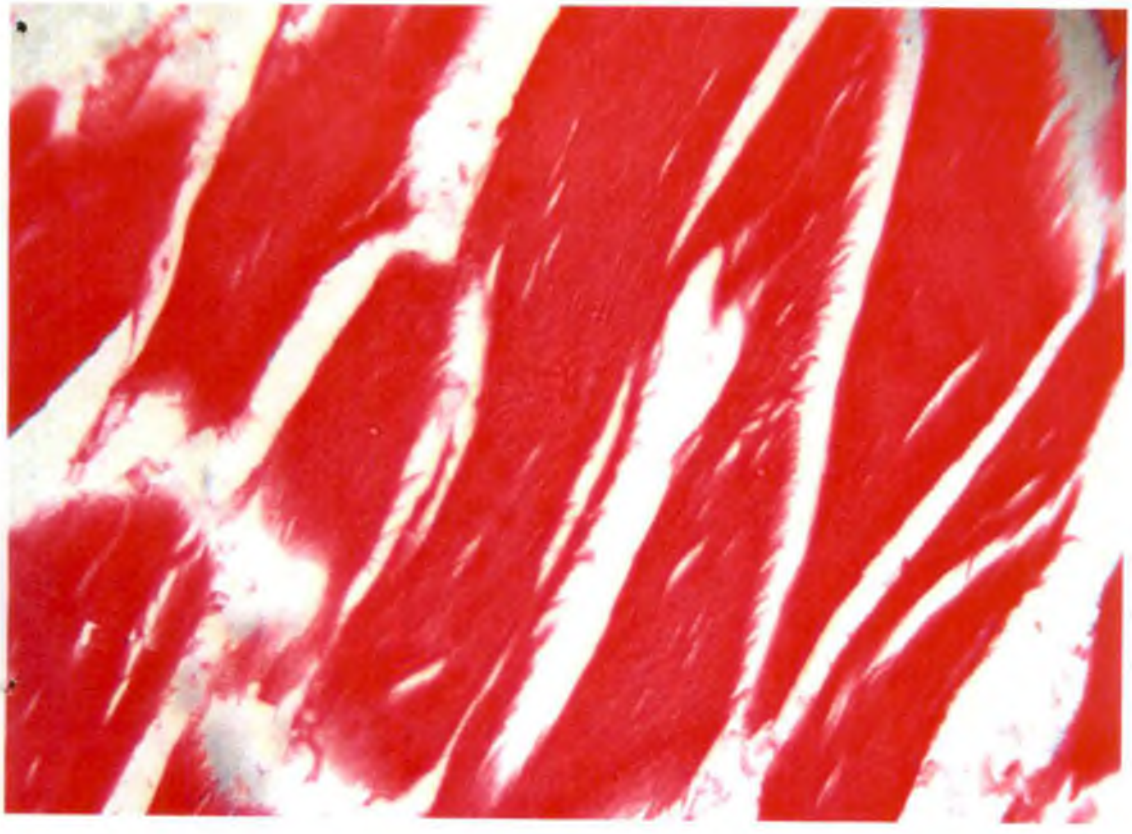


Plate 16. Longitudinal section of breast muscle of ethinylestradiol +levanorgestrel and alpha-tocopherol fed group (G-IV)

Discussion

5. DISCUSSION

5.1 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON PRODUCTION PARAMETERS

5.1.1 Body weight

The body weight of birds in various groups did not differ significantly through out the experimental period (table 3).

Rumsey *et al.* (1990) suggested that a measurable response to estrogenic implants could occur only over the dry matter intake range above maintenance intake of adequately balanced diets as estrogen is found to increase the BMR. Mader, T.L. (1994) reported that delaying estrogen administration till the animal has reached late growing period or using a low dose implant during an initial feeding period provides performance enhancement in subsequent feeding periods. He also noted that high dose implants of estrogen have more effect on weight gain during the growing phase than the low dose implants. Both the above may explain why the ethinylestradiol+levanorgestrel supplementation failed to elicit any response in bodyweight of broiler chicken as the duration of exposure was shorter (only four weeks) and time of exposure was the late growing and finisher phase. Generally in farm animals' estrogen is found to increase final body weight. According to Hutcheson *et al.* (1992) live weight (14 percent) and carcass weight (12 percent) were greater in implanted lambs compared to controls.

Few authors also had similar observations in the farm animals as in the present study. Kniffen *et al.* (1999) observed no difference in body weight and carcass traits during growing as well as finishing phases of heifers implanted with estrogen. They also found that the relative growth rate tended to reduce both in single and double implanted heifers.

While it is difficult to explain why α-tocopherol supplementation failed to improve growth and final body weight as the general notion is just the reverse.

Supplementation of α-tocopherol significantly improved growth in broiler chicken (Lin *et al.,* 1989). Vitamin E at level of 250 mg/kg significantly improved final bodyweight between the supplemented group and control in cold stressed layers (Kucuk *et al.,* 2003). Al-Taleb (2003) observed that birds fed the diet supplemented with 2.5ml/l vitamin AD_3E + 15 g vitamin C were significantly ($P<0.05$) heavier than the rest of the birds. While there are reports in farm animals in which authors had views similar to the results of the present study. Cannon *et al.,* (1996) observed no effect of vitamin E at 100 mg/kg of feed on growth, carcass characteristics such as dressing percentage and shrinkage and proximate composition of pigs.

5.1.2 Average daily gain (ADG)

Alpha-tocopherol alone or in combination with ethinylestradiol +levanorgestrel resulted in significant improvement in ADG in broiler birds (table 3). Conflicting reports are available regarding the effectiveness of α-tocopherol in improving average daily gain. Live weight gain increased in birds linearly in birds as the dietary vitamin E increased up to 250 mg/kg and further increase up to 500 mg/kg didn't cause an increase (Sahin *et al.,* 2002). Feeding lambs with 500 IU of vitamin E over a period of 56 days improved daily gain (Wulf *et al.,* 1995). According to Soler –Velasquez *et al.,* (1998) supplemental Vitamin E increased average daily gain in finisher swine. Weight gain of chicks during 0 – 3 weeks of age was not affected by dietary vitamin E levels of 10 to 20 mg/kg (Hsieh *et al.,* 2002). No differences were apparent for initial weight, average daily gain during the starter or grower phases of pigs fed corn-soybean meal based diets (Lepine *et al.,* 1990). The addition of α-tocopheryl acetate had no effect on average daily gain of pigs (Dove and Ewan. 1991; Anderson *et al.,* 1995).

Ethinylestradiol+levanorgestrel failed to elicit any response. This finding is contradictory to the finding in farm animals where estrogen is belived to improve ADG while there are no literature available in birds to comment on. Average daily gain improved in response to estradiol in steers (Enright *et al.,* 1990). Estradiol-17*β*

implants increased weight gain in steers while they did not increase weight gain in bulls. Moran *et al.* (1991) found that zeranol implants in heifers caused an increase of average daily gain to the extent of 8.9%. According to Hutcheson *et al.* (1992) average daily gain was greater in implanted lambs compared to controls. Preston *et al.* (1995) reported increase in average daily gain and gain efficiency when feed lot steers were implanted with estradiol benzoate, progesterone and trenbolone acetate combination.

5.1.3 Feed efficiency (FE)

Both ethinylestradiol+levanorgestrel and α-tocopherol alone or in combination failed to influence the feed efficiency in the experimental birds (table 4). Similarly Hunt *et al.* (1991) observed that estradiol implants in steers had no effect on feed efficiency. Lee et al (1990) found out that Estradiol-17 *ß* implants didn't change feed intake in steers and bulls. There are also evidences about the ability of estrogen preparations to improve feed intake and efficiency. According to Enright *et al.* (1990) Estradiol increased feed conversion efficiency in steers. Rumsey *et al.* (1992) observed a trend for increase in dry matter intake and feed conversion in steers treated with implants containing estradiol benzoate and Progesterone. They found that the intake:gain ratio was also improved by the implants.

In the present investigation α-tocopherol failed to evoke any improvement in feed efficiency in broiler birds. Results of the present investigation was some what similar to few published works in broiler birds and farm animals. Gain:feed ratio of chicks during 0 – 3 weeks of age were not affected by dietary vitamin E levels of 10 to 20 mg/kg (Hsieh *et al.*, 2002). Lepine *et al.* (1990), Dove and Ewan. (1991), Cannon *et al.* (1996) and Waylan *et al.* (2002) found that vitamin E had no influence on gain:feed ratio in swine. There are also reports available in which authors had suggested a positive impact of vitamin E in birds and farm animals. Feed efficiency increased in birds subjected to heat stress linearly as the dietary vitamin E increased

up to 250 mg/kg and further increase up to 500 mg/kg didn't increase the feed efficiency (Sahin *et al.*, 2002). Kucuk *et al.* (2003) noted significant improvement in feed efficiency between the Vitamin E (250 mg/kg) supplemented group and control in cold stressed layers. Kennedy *et al.* (1992) reported that greater level of vitamin E supplement could enhance productivity as a result of improvement in both feed conversion efficiency and higher average weight gain in broiler chicken.

5.2 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON SLAUGHTER TRAITS

5.2.1 Dressing percentage

Dressing percentage was significantly improved in the present experiment by both ethinylestradiol+levanorgestrel and α-tocopherol when they were given alone in birds (table 3). Data regarding effectiveness of ethinylestradiol+levanorgestrel and α-tocopherol in birds are inconclusive while most of the data available belongs to farm animals. Commercial implant Synovex S (Estradiol benzoate and Trenbolone acetate) in beef cattle increased the dressing percentage (Platter *et al.*, 2003). While some authors had a differing opinion regarding the effectiveness of estrogen preparations in farm animals. According to Hutcheson *et al.* (1992) Zeranol showed no influence on dressing percentage among lambs. Cannon *et al.*, (1996) observed no effect of vitamin E at 100 mg/kg of feed on dressing percentage. However, Rivera *et al.* (2002) reported that vitamin E supplementation showed a higher dressing percentage in heifers.

5.3. EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON HAEMATOLOGICAL PARAMETERS

Haematological evaluation can help in ascertaining overall health status of an animal in the sense that drastic variations in haematological parameters from physiological range reflect deviation from normal health (tables 6a and 6b).

5.3.1 Total erythrocyte count (TEC), Haemoglobin (Hb) concentration, Volume of packed red cells (VPRC) and Erythrocyte indices

In this study at sixth and eighth week of age Ethinylestradiol+levanorgestrel supplementation significantly lowered (P>0.05) TEC in birds of G-II group when compared to G-I, G-III and G-IV groups (table 5a). The Hb value was significantly lowered (8.75 ± 0.19 g%) by the supplementation of ethinylestradiol+levanorgestrel at eighth week of age. α-tocopherol supplementation for a longer period (eighth week of age) had a positive effect on Hb value both in the α-tocopherol fed group G-III and birds fed with combination ethinnylestradiol+levanorgestrel and α-tocopherol (G-IV) and the values were higher (9.75 ± 0.23 g% and 10.31 ± 0.16 g% respectively). In the present experiment ethinyloestadiol+levanorgestrel treatment significantly reduced the value of VPRC in G-II group of birds with respect to age and when compared to other treatments (table 5a).

The pattern observed in MCV among groups is due to higher initial value observed in the ethinylestradiol+levanorgestrel group G-II. The only significant change (lowering) value is observed in G-II from the fourth week value (92.49 ± 3.86 fl) to sixth week (81.17 ± 1.56 fl) which proved the effect of estrogen (table 5b).

At sixth week of age in the birds in groups G-II, G-III and G-IV had significantly lower MCH value (P<0.05) than that of control group (G-I). That trend reversed in the eighth week of age where both G-I and G-II had significantly lower values (P<0.05) than the birds in G-III and G-IV (table 5b). Dietary supplementation of α-tocopherol and combined supplementation of ethinnylestradiol+levanorgestrel and α-tocopherol had significant influence on the MCH values in both G-III and G-IV groups.

Exactly similar trend could be observed in MCHC values as that observed in MCH value (table 5b). At sixth week the values in all treatment groups were lower when compared to control gorup G-I at slaughter (eighth week of age) birds in both α-tocopherol fed group and birds fed with combination of

ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) had significantly higher MCHC values when compared to others which again demonstrated the positive effect of α-tocopherol which was also evident as the birds grew older.

The available literature to explain this particular effect in broiler chicken is scanty. The salient features of our results were in agreement with findings of Nirmalan and Robinson (1972) whom observed significantly lower erythrocyte counts, packed cell volume and hemoglobin concentration in exogenous oestrogen analogue (Stilbestrol) treated quails. The possible explanation could be the decrease of haemopoietic marrow in long bones under the influence of estrodiol as explained by Field *et al.* (1990). The positive effect shown by vitmain E on the concentration of Hb and erythrocyte indices may be the result of its ability to protect haemopoietic tissue from oxidative damage.

5.3.2 Total leucocyte count (TLC)

There was an uncharacteristic lowering of TLC in α-tocopherol fed group at sixth week of age and it just reversed to the highest values among the groups at eighth week of age (table 5a). TLC value in ethinylestradiol+levanorgestrel group always remained low during treatment. This indicated that ethinylestradiol+levanorgestrel had a lowering effect on TLC while α-tocopherol had an enhancing effect. The lowering effect of ethinylestradiol+levanorgestrel fed group may be the higher progestationl compound contained in the preparation, as progesterone at higher doses is immunosuppressive (Tizard 1996). While the findings in the study are contradictory to the observations of Luster *et al.* (1984) and Ahmed *et al.* (1989) where in they have suggested an immune cell enhancing effect for estrogen. A hoard of authors had suggested that vitamin E has the immune function enhancing property, Erf *et al.* (1997) in broiler chicken, Tengerdy. (1989) and Field *et al.* (2002) in laboratory animals, farm animals, and humans, which could explain the result obtained in group G-III (α-tocopherol fed) and G-IV (ethinylestradiol+levanorgestrel and α-tocopherol).

5.4 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON BIOCHEMICAL PARAMETERS

5.4.1 Plasma protein profile

5.4.1.1 Total proteins

In the present study the changes in the plasma concentration of total protein was insignificant at the fourth and eighth week of age (table 6). There was significant difference at the sixth week especially between birds in α-tocopherol group and rest of the birds. The value was lower than the control as well as rest of the birds. This was uncharacteristic in such a way that most of the previous studies had indicated that vitamin E supplementation would probably result in improvement in total protein level (Sahin *et al.,* 2002). While our results were similar to the one observed by Arslan *et al.* (2001) where they found no significant difference in total protein between control and α-tocopherol supplemented birds. Our study shows that ethinylestradiol+levanorgestrel has no effect on total protein levels. There was no significant influence of age on the total plasma protein concentration at fourth, sixth and eighth week of age in birds of control group G-I and groups G-II, G-III, and G-IV. Similar observations were made by Arslan *et al.* (2001) where in which they found no significant difference in concentration of plasma total protein between control and vitamin E supplemented birds by the fifth and seventh week of age.

5.4.1.2 Albumin

Dietary ethinylestradiol+levanorgestrel and α-tocopherol supplementation significantly ($P<0.05$) lowered the plasma albumin concentration in birds of group G-III while the highest value was recorded in G-II birds (table 6). While at eighth week the lowest value was recorded in the control group, G-I. Similar findings are not available to make comment on this observation. While Shin *et al,* (2002) observed significant increase in albumin level in birds supplemented with vitamin E at 250 mg/kg in the ration.

5.4.1.3 Globulin

There was no significant difference in plasma globulin levels between the diferent groups (table 6). The results showed that both α-tocopherol and ethinylestradiol+levanorgestrel alone or in combination failed to influence the globulin levels in broiler chicken. The above results has serious repercussions such that the humoral immunity stimulating property of the vitamin E as suggested by authors such as Rivera *et al.* (2002) are being doubtfull. The authors suggested an increase in circulating antibodies to foreign antigen noted with supplementation of 1140 IU/d indicates that vitamin E can enhance humoral immune response in cattle. Few authors like Hatfield *et al.* (2002) recorded that vitamin E supplementation in yearling ewes (0 to 330 IU per ewe per day) failed to influence humoral immune response. Tengerdy (1989) and Field *et al.* (2002) suggested that vitamin E as a dietary supplement or as part of an adjuvant vaccine formulation increases humoral and cell-mediated immunity and disease resistance in laboratory animals, farm animals, and humans.

The observations recorded in this study were similar to the one observed by Reffet *et al.* (1988) and Daniels *et al.* (2000) in lambs where supplemented vitamin E found to have no effect on IgG levels.

The results recorded in the estrogen exposed group also counters the arguments made by Ahmed *et al*, (1989) wherein the authors suggested that the overall augmented humoral immune responses in females appears to be due to estrogen. Similarly, Leitner *et al.* (1996) also noted that administration of estradiol 3-benzoate enhanced significantly the humoral immune response to *Escherichia coli* and sheep erythrocytes.

5.4.1.4 Albumin: Globulin ratio

At fourth, sixth and eighth week of age there was no significant effect of treatments on A:G ratio of broiler chicken (table 6). The present results may indicate

the failure of ethinylostradiol+levanorgestrel and α-tocopherol alone or in combination to influence protein production in liver of broiler birds.

5.4.2 Plasma lipid profile

5.4.2.1 Total cholesterol

At sixth week of age dietary ethinylestradiol+levanorgestrel supplemented group G-II showed a significantly ($P<0.05$) lower plasma cholesterol concentration (122.09 ± 3.94 mg/dl) when compared to group G-IV fed with a combination of ethinnylestradiol+levanorgestrel and α-tocopherol (table 7a and fig. 3). Similarly at eighth week of age significantly ($P<0.05$) the lowest cholesterol concentration was observed in birds of ethinylestradiol+levanorgestrel supplemented group G-II (103.67 ± 6.26 mg/dl) when compared to rest of the groups G-I, G-III, G-IV (133.57 ± 4.26 mg/dl, 126.07 ± 4.34 mg/dl and 123.74 ± 7.41 mg/dl respectively). There was no significant variation in cholesterol concentration between groups G-I, G-III and G-IV.

Among the periods, birds in ethinylestradiol+levanorgestrel supplemented group had shown significant reduction ($P<0.05$) in cholesterol concentration from fourth to eighth week of age while rest of the experimental groups showed no significant change.

The results obtained suggest that the ethinylestradiol+levanorgestrel supplementation could decrease the plasma cholesterol concentration. Similar estrogen associated reduction in total plasma cholesterol was observed in several animal studies (Williams *et al.,* 1990, Haarbo *et al.,* 1991, Bjarnason *et al.,* 1997, Adams *et al.,* 1997). Oral combined hormone replacement therapy (HRT) with estradiol and norethisterone resulted in a 12% decrease in total cholesterol (Peverill *et al.,* 2001). Koh *et al.* (2003) also opined that a combination of conjugated equine estrogen and progesterone significantly reduced total cholesterol in postmenopausal woman.

The lowering of plasma total cholesterol may be due to the enhanced catabolism of cholesterol components in liver. There is also evidence of receptor up-regulation by various estrogens leading to enhanced clearance of cholesterol components from circulation.

The literature also indicated cases where the results obtained are against to the observations in the present study. Park *et al.* (1988) showed that estradiol implants in chicks resulted in marked elevation of all major plasma lipids with the increase in concentration of cholesterol. Similarly Cho *et al.* (1988) showed that young male chickens implanted with estrogen for three week developed a marked four fold increase in cholesterol, over controls. Few authors also suggested that estrogen supplementation could result in an increase in cholesterol level in animals (Wagner *et al.,* 1991 and 1997, Sulistiyani *et al.,* 1995, Hanke *et al.,* 1996, Haines *et al.,* 1999) Compared with controls, a significant increase was observed in the plasma levels of cholesterol (8–16 %) during treatment with formulation containing ethinyl estradiol and gestodene/norgestimate (Wiegratz *et al.,* 1998).

Endrikat *et al.,* 2002 sugested that oral contraceptives containing ethinylestradiol and levanorgestrel caused an increase in total cholesterol in humans. Monophasic oral contraceptives containing ethinylestradiol (20 μg or 3o μg) and levanorgestrel (100 μg or 150 μg) caused slight increase in total cholesterol in treated women (Scharnagl *et al.,* 2004).

There are also reports in which supplemental estrogen failed to evoke any effect. Zandberg *et al.* (1998) suggested that neither subcutaneous estradiol deconate nor oral 17-*ß* estradiol could not affect the mean plasma cholesterol concentration in overiectamized rabbits. Conjugated equine estrogen showed no effect on total cholesterol in postmenopausal women (de Valk-de Roo *et al.,* 1999).

Results obtained in the present study indicated that α-tocopherol supplementation may not be able to affect the total plasma cholesterol level. The results are similar to the findings of Donaldson (1982) and Smith and Kummerow

(1989) where they observed no significant effect of dietary vitamin E supplementation on the plasma cholesterol level in broiler chicken. In another study conducted by Arslan *et al.* (2001) observed no significant difference in plasma cholesterol, concentration between control and vitamin E supplemented birds by the fifth and seventh weeks of age. Lepine *et al.* (1990), Wuryastuti *et al.* (1993) and Soler –Velasquez *et al.* (1998) had also mentioned similar observations in swine supplemented with vitamin E. Salvatori *et al.,* 2004 also had obtained similar results in cross bred lambs administered intramuscular dosage (200 IU) of vitamin E. But there are few reports in which it is suggested that vitamin E did have its effects. Franchini *et al.,* (1988) also reported that the supplementation of vitamin E at a dosage of 325 ppm resulted in a decrease in the level of cholesterol and the decrease is related to age and was definite by day 49 in broilr chicken. Franchini *et al.* (1990) also reported that cholesterol level of turkeys fed with a vitamin E supplemented diet lowered the plasma cholesterol level on the day 42 and it reached its maximum level on the day 86. Sahin *et al.* (2002) and Kucuk *et al.* (2003) observed significant reduction in serum cholesterol concentrations in heat stressed and cold stressed layers supplemented with 250mg/kg vitamin E.

The possible explanation to the results obtained in the present study could be that the estrogen supplementation could have resulted in accelerated clearance of cholesterol component from the circulation. This can be attributed to enhanced receptor activity in target tissue courtesy of estrogen activity in the respective tissues (Lansink *et al.,* 1999).

5.4.2.2 High density lipoprotein (HDL)

In the present study ethinylestradiol+levanorgestrel and α-tocopherol either alone or in combination significantly increased HDL levels in birds from sixth to eighth week of age table 7b and fig. 4. There was also age related increase in all the treatment groups (G-II, G-III, G-IV). This increase may be due to enhanced synthesis of HDL by liver in response to estrogen administration or suppressing

hepatic lipase. The results also suggested the prominence of estrogen component since the progestogens has a tendency to upregulate hepatic lipase and hence the breakdown of HDL. The results of the study were similar to Meade *et al.* (1977) and Fotherby and Caldwell (1994) who observed in healthy women. Ethinylestradiol increases the concentration of HDL (Tikkanen and Nikkila., 1986; Krauss and Burkman., 1992; Machado *et al.,* 2004) in the plasma.

Conjugated equine estrogen increased HDL concentration from six months of treatment in postmenopausal women (de Valk-de Roo *et al.,* 1999). A combination of conjugated equine estrogen and progesterone significantly increased the levels of triglyceride and HDL cholesterol in postmenopausal woman (Koh *et al.,* 2003). Oral contraceptives containing ethinylestradiol and either desogestrel or cyproterone acetate increased the level of total cholesterol, LDL cholesterol and HDL cholesterol in adolescents with polycystic ovarian syndrome (PCOS) (Mastorakos *et al.,* 2002) and in male Wistar rats (Kamisako and Ogawa., 2003).

Pronounced decrease in HDL_2 cholesterol was observed in the ethinyl estradiol and levanorgestrel in exposed humans (Tikkanen *et al.*, 1982, Kuusi *et al.,* 1985). Similarly, oral contraceptives containing ethinylestradiol in combination with the progestogen levonorgestrel tend to lower HDL and HDL2 in particular (Notelovitz *et al.*, 1989; Janaud *et al.,* 1992; Endrikat *et al.,* 2002). Combination of ethinylestradiol with higher doses of levanorgestrel may cause a decrease in the level of HDL cholesterol (Crook and Godsland. 1998; Scharnagal, *et al.,* 2004).

5.4.2.3 Low density lipoprotein (LDL)

At fourth week of age within groups there was no significant variation in the level of plasma LDL (table 7b and fig. 5). There was significant ($P<0.05$) lowering in plasma LDL value in the ethinylestradiol+levanorgestrel group G-II when compared to the control group G-I at sixth week of age. The LDL value in other groups did not differ significantly. At eighth week of age significantly lower LDL value was observed in the ethinylestradiol+levanorgestrel group G-II. It had the

lowest value (25.80 ± 7.15 mg/dl) than the other groups. The birds of control group G-I had the highest value of 70.53 ± 4.68 mg/dl. Alpha-tocopherol fed group G-III and birds fed with combination of ethinnylestradiol+levanorgestrel and α-tocopherol (G-IV) had significantly ($P<0.05$) lower value of 46.97 ± 2.19 mg/dl and 45.73 ± 5.82 mg/dl respectively when compared to control group G-I. Birds of both G-III and G-IV groups also had significantly ($P<0.05$) higher values when compared to ethinylestradiol+levanorgestrel fed group G-II.

Among the periods, the plasma LDL value showed significant ($P<0.05$) association with age in ethinylestradiol+levanorgestrel fed group (G-II), α-tocopherol fed group (G-III) and birds fed with combination of ethinnylestradiol+levanorgestrel and α-tocopherol (G-IV). In these three groups significantly lowest values ($P<0.05$) were recorded at the eighth week of age when compared to fourth week. In all the three groups the trend was similar.

The LDL lowering effect shown by ethinylestradiol+levanorgestrel may be due to the LDL receptor enhancing action of estrogens in the target tissues, which may result in higher clearance of LDL from circulation. This may be in accordance with findings of Schaefer *et al.* (1983) and Machado *et al.* (2004) who observed that treatment with potent estrogens decreases the plasma concentration of LDL in exposed women. While esterified estrogens were reported to cause reduction of LDL in cyanomegalous monkey (Wagner *et al.*, 1996). The lowered LDL may enhance the atheroprotective effect due to lesser deposition of LDL. Similar findings are reported by Walsh *et al.* (2000) where a 50% decrease in basal LDL accumulation rate in arteries was recorded from estradiol treated animals. Conjugated equine estrogen reduced LDL in postmenopausal women (de Valk-de Roo *et al.*, 1999). A combination of conjugated equine estrogen and progesterone significantly reduced LDL cholesterol levels in postmenopausal woman (Koh *et al.*, 2003). Oral combined hormone replacement therapy (HRT) with estradiol and norethisterone resulted in a 11% decrease in LDL cholesterol (Peverill *et al.*, 2001). Oral estrogen administration

reduces low density lipoprotein (LDL) cholesterol (Sacks *et al.,* 1994, Leung *et al.,* 2004).

The results obtained in this study differed from the findings of few authors who suggested an increase in LDL level upon exposure to estrogens. Oral contraceptives containing ethinylestradiol and levanorgestrel caused increase in LDL cholesterol (Endrikat *et al.,* 2002). Scharnagl *et al.* (2004) also observed a slight increase in LDL cholesterol in women treated with monophasic oral contraceptives containing ethinylestradiol (20 μg or 3o μg) and levanorgestrel (100 μg or 150 μg). Oral contraceptives containing ethinylestradiol in combination with the progestogen levonorgestrel tend to raise LDL concentration (Notelovitz *et al.*, 1989; Janaud *et al.,* 1992). Combination of ethinylestradiol with higher doses of levanorgestrel causes an increase in LDL cholesterol (Crook and Godsland. 1998; Scharnagal, *et al.,* 2004).

5.4.2.4 Very low density lipoprotein (VLDL)

Effect of ethinylestradiol+levanorgestrel and α-tocopherol on plasma VLDL in the four treatment groups at fourth, sixth and eight week of age are given in table 7b and fig. 6. At fourth week of age there was significant difference ($P<0.05$) in plasma VLDL value in ethinylestradiol+levanorgestrel fed group G-II (highest) and the rest of the groups G-I (lowest), G-III and G-IV. Birds of all the three treatment groups (G-II, G-III, and G-IV) had significantly lower ($P<0.05$) VLDL values than the control group at sixth week of age. At eighth week of age the trend in VLDL values were similar to the one found in the sixth week. The exception was only in the lowest value, which was observed in the α-tocopherol fed group rather than the ethinylestradiol+levanorgestrel fed group, as was the case in sixth week. There was significant difference ($P<0.05$) in VLDL value in the control group and the rest of the groups.

Among the periods, the VLDL concentration remained same in birds of G-I group through out the experiment. In all other groups the level of VLDL showed a significant reduction from fourth to sixth week of age and then the trend continued

till the end. In birds of all three treatments there was a drastic reduction in the VLDL values through the age.

There was considerable lowering of the VLDL values in the ethinylestradiol+levanorgestrel, α-tocopherol and those fed with combination of ethinnylestradiol+levanorgestrel and α-tocopherol throughout the experiment period. Both estrogen and α-tocopherol alone or in combination caused reduction in plasma VLDL values. This may be due to higher turn over of VLDL in hepatocytes. Other wise it could be due to progestational compound (levanorgestrel) which could oppose the metabolic effects of estrogen on lipids (Meade *et al.*, 1977 and Fotherby and Caldwell. 1994). Similar results are not found in literature with respect to avian species. The results of the study were in accordance with Wagner *et al.* (1996), who suggested that esterified estrogen with and without methyl testosterone lowered total plasma VLDL in cynomegalous monkeys challenged with atherogenic diet. While few others had a different opinion such that they claimed that the estrogen administration could raise the VLDL level. This could be due to enhanced production of VLDL from hepatocytes (Tikkanen and Nikkila., 1986 and Krauss and Burkman., 1992). The results of study conducted by Campos *et al.* (1997), showed that the oral administration of 2 mg estradiol 17ß daily, given in the micronized form for 6 weeks associated with an increase in light, or buoyant, very low density lipoprotein (VLDL) in postmenopausal women. Oral contraceptives containing ethinylestradiol and levanorgestrel caused increase in VLDL cholesterol in humans (Endrikat *et al.*, 2002).

There are also observations in which neither subcutaneous estradiol deconate nor oral 17-*β* estradiol affected the mean VLDL concentration in overiectamized rabbits (Zandberg *et al.*, 1998).

5.4.2.5 Triglycerides

At fourth week there was significant difference ($P<0.05$) in triglycerides concentration among the birds of group G-II and rest of the groups with lowest value

recorded in birds of control group G-I and the highest value in birds of ethinylestradiol+levanorgestrel fed group G-II (table 7a and fig. 7). There was significant variation ($P<0.05$) in triglycerides concentration between control (G-I) and all the treatment groups (G-II, G-III and G-IV) at sixth week of age. The control group had the highest (109.73 ± 1.77 mg/dl) triglycerides concentration when compared to that of treatment groups G-II, G-III and G-IV. There was significant influence ($P<0.05$) of age on triglycerides values of treatment groups (G-II, G-III and G-IV) except in the control group G-I with drastic decline in triglyceride values with advance in age with highest at fourth and lowest at eighth week.

The results of the study suggest that the estrogen as well as α-tocopherol either alone or in combination caused reduction in plasma triglyceride levels in broiler chicken. The possible mechanism may be the interference with hepatic synthesis of triglycerides. Similar findings are not observed in birds in the available literature. The results were some what similar to the findings of Wiegratz *et al.* (2002) where formulations containing ethinylestradiol and levanorgestrel did not change the triglycerides level in women. While majority of the literature suggested that estrogen exposure results in increase in triglycerides in broiler chicken as well as in other animals. Treatment with potent estrogens increases the plasma concentrations of TG (Schaefer *et al.,* 1983; Machado *et al.,* 2004). Park *et al.* (1988) showed that Estradiol implants in chicks resulted in marked elevation in triglycerides (TG). The results of Cho *et al.* (1988) showed that young male chickens implanted with estrogen for three weeks developed a marked hypertriglyceridemia, and recorded a 68 fold increase over control birds.

Cho and Park (1990) compared estrogen treatment in fed birds as well as fasted birds with its control resulted in a marked elevation of triglyceride during the 2-day period. Even in chicks fasted for 5 days; estrogen treatment resulted in a persistent hypertriglyceridemia. In fed chicks, estrogen treatment also induced a fatty

liver with massive accumulation of triglyceride, but the liver of estrogen treated-fasted chicks appeared to be normal.

With regard to effect of α-tocopherol the results were similar to Franchini *et al.*, (1988) who reported that the supplementation of vitamin E in broiler chicken at a dosage of 325 ppm resulted in a decrease in triglyceride levels related to age and was definite by day 49. With an increase in dietary vitamin E, serum triglycerides concentration reduced linearly in birds exposed to heat stress (Sahin *et al.*, 2002). Kucuk *et al.* (2003) also observed significant reduction in serum triglycerides concentrations in cold stressed layers supplemented with 250mg/kg vitamin E. Franchini *et al.* (1990) found that increasing the levels of vitamin E lowered the level of triglyceride on the day 28, and on day 42 it increased to the initial level.

Some of the authors also had a differing point of view where they argue that the vitamin E had no role in triglyceride metabolism. Dietary vitamin E supplementation has no significant effect on cholesterol level in broiler chicken (Donaldson 1982; Smith and Kummerow 1989). Arslan *et al.* (2001) observed no significant difference in plasma concentration of triglycerides between control and vitamin E supplemented birds by the fifth and seventh weeks of age. In weanling pigs vitamin E at levels of 20, 40, 60 and 100 IU/kg diet did not produce any effect on the serum triglyceride concentration (Moreira and Mahan, 2002).

5.4.2.5 Total lipids

Effect of the ethinylestradiol+levanorgestrel and α-tocopherol treatment could be observed at sixth week age, where the birds of α-tocopherol fed group G-III had significantly ($P<0.05$) higher plasma total lipids concentration when compared to the control group G-I, ethinylestradiol+levanorgestrel fed group G-II, which was the lowest and group G-IV fed with combination of ethinnylestradiol+levanorgestrel and α-tocopherol (table 7a and fig. 8). The ethinylestradiol+levanorgestrel fed group G-II had significantly ($P<0.05$) lower total lipid concentration when compared to that of groups G-1, G-III and G-IV at sixth and eighth week of age. Among the periods, the

total lipid concentration was significantly ($P<0.05$) higher in birds of α-tocopherol fed group G-III at sixth and eighth week when compared to fourth week. Both G-I and G-III group recorded reduction in their values by eighth week compared to sixth week values. Whereas in ethinylestradiol+levanorgestrel treated group (G-II) there was significant ($P<0.05$) decrease in total lipids with advancing age. The group G-IV also showed similar trend but was non significant.

The results suggested that the ethinylestradiol+levanorgestrel supplementation has lipid lowering effect in birds. The exact mechanism for such could not be found in the available literature. It could be due to enhanced metabolism of lipids in the hepatocytes. Most of the literature available suggested an increase in plasma total lipids under the influence of estrogen with one or more of the lipid component showed an increase in their concentration. Park *et al.* (1988) showed that Estradiol implants in chicks resulted in marked elevation of all major plasma lipids with greatest increase in triglycerides (TG) followed by phospholipid (PL). The results of Cho *et al.* (1988) showed that young male chickens implanted with estrogen for three week developed a marked hyperlipidemia. Plasma levels of triglyceride, and phospholipid were elevated 68 and 24-fold, respectively, over controls. Cho and Park (1990) compared estrogen treatment in fed birds as well as fasted birds with its control resulted in a marked elevation of plasma lipids, especially the levels of triglycerides during the 2-day period. Even in chicks fasted for 5 days; estrogen treatment resulted in a persistent hypertriglyceridemia.

The significantly higher levels of total lipids at sixth week in α-tocopherol fed group and there after its reduction to values similar to fourth week is difficult to explain as there is no evidence of such change which are not found in the available literature.

5.4.3 Plasma enzyme profile

5.4.3.1 Gamma glutamyltransferase (GGT)

Effect of treatment was observed only at sixth week of age with significant difference ($P<0.05$) in GGT level of ethinylestradiol+levanorgestrel group G-II with rest of the groups G-I, G-III and G-IV (table 8 and fig. 9). The birds of ethinylestradiol+levanorgestrel fed group G-II had the highest (23.25 ± 0.86 U/l) and the birds of α-tocopherol supplemented group G-III had the lowest (14.87 ± 1.03 U/l) GGT levels. Among the periods there was significant influence ($P<0.05$) of age in plasma GGT concentration of birds of ethinylestradiol+levanorgestrel group fed (G-II), which showed drastic increase in GGT values with age. A non significant reduction in GGT value was observed in α-tocopherol group and birds fed with combination of ethinnylestradiol+levanorgestrel and α-tocopherol (G-III and G-IV) at sixth week of age when compared to the values observed at fourth and eighth week of age.

Lim *et al.* (2004) suggested that serum γ-glutamyltransferase (GGT) level within normal range may act as an early marker of oxidative stress in humans. They also noted that vitamin E was not associated with serum GGT level. The drastic increase in GGT may be due to the cholestatic effect of ethinylestradiol. Ethinylestradiol showed cholestatic effect in rats (Davis and Kern., 1976; Rodriquez *et al.,* 1992; Bossard *et al.,* 1993; Puglielli *et al.,* 1994; Simon *et al.,* 1996 and Geier *et al.,* 2003) and also the fact that GGT shows an increase in conditions of obstructive cholestasis. While Dahlgren *et al.* (1998) observed no derangement of liver enzymes in the women treated with ethinylestradiol and cyproterone acetate.

5.4.3.2 Aspartate aminotransferase (AST)

The plasma AST also showed similar trend as that of GGT with drastic increase ($P<0.05$) in values at sixth and eighth week of age in the ethinylestradiol+levanorgestrel treated group (table 8 and fig. 10). At eighth week of age α-tocopherol (G-III) and birds fed with a combination of

ethinylestradiol+levanorgestrel and α-tocopherol group (G-IV) had significantly ($P<0.05$) higher plasma AST concentration when compared to plasma AST values of control group G-I.

As the influence of age was compared the values of plasma AST at eighth week, the values were found to be significantly ($P<0.05$) higher in all the experiment groups except the control group G-I. Plasma AST values showed significant increase in ethinylestradiol+levanorgestrel fed group (G-II) from fourth to sixth week of age and slight reduction in eight week

Similar to the earlier discussion regarding the role of ethinylestradiol+levanorgestrel in elevating GGT, the increase in AST value could also be attributed to cholestasis or other damaging effects. The available literature does not provide scope for much deliberation in this regard.

The increase in plasma AST value in G-III and G-IV groups at eighth week of age was contradictory to the response found in most of the available literature where it is expected to show either a decline or no effect at al. Somewhat similar observations could be found in observations made by Franchini *et al.,* (1990) where AST level increased together with vitamin E in young turkeys, while such trend was not found in hens. This finding with regard to hens was similar to his earlier findings in broiler chicken (Franchini *et al.,* 1988) where there was a non significant change between the control and experimental groups with respect to AST. Arslan *et al.* (2001) observed no significant difference in the level of AST between control and vitamin E (100, 200 and 300 ppm) supplemented birds by the fifth and seventh week of age. Serum activity of AST was not influenced by dietary vitamin E supplementation in birds subjected to heat stress (Sahin *et al.,* 2002). Regarding age related increase it may be attributed as a side effect of excessive metabolism that one could come across during the rapid growing phase of bird such as broilers.

5.4.4 Effect of ethinylestradiol+levanorgestrel and α-tocopherol on plasma antioxidant status

Antioxidant status of animals is usually assayed by estimating the activity of free radical scavenging enzymes like superoxide dismutase (SOD), catalase and also through measuring the peroxidation levels. In the present investigation assessment of antioxidant status of the chicken has been confined to SOD, catalase activity and lipid peroxidation levels.

5.4.4.1 Superoxide desmutase (SOD)

There is meager literature available to explain the effects of ethinylestradiol+levanorgestrel and α-tocopherol on the effect of SOD level in plasma (table 9 and fig. 11). Both had significant effect at sixth week of age when compared with treatments, while the declining trend in levels from fourth to eighth week indicate that all treatments failed to elicit any response. In this context the results of present study were similar to findings of few authors such as Walsh *et al.* (1993) who observed that vitamin E did not control SOD activity in muscles of calves, depleted of vitamin E. In turkey meat vitamin E supplementation had no effect on SOD activity (Renerre *et al.*, 1999). While, Gatellier *et al.* (2004) observed a highly significant correlation between SOD activity and vitamin E content of muscles in cattle ($r = 0:591$; $p < 0:001$).

5.4.4.2 Catalase

In the present study α-tocopherol alone or when combined with ethinylestradiol+levanorgestrel resulted in a significant increase in catalse activity (table 9 and fig. 12). This was observed only at sixth week of age. Among the periods there was significant influence ($P<0.05$) of age in plasma catalase activity of birds in all the four groups with an increase in activity by the sixth week. The increase was more prominent in α-tocopherol fed group (G-III) and birds fed with a combination of ethinylestradiol+levanorgestrel and α-tocopherol (G-IV). The entire groups showed a marginal reduction in catalase activity by the eighth week of age.

The change in catalase activity with age was lesser in control group (G-I) when compared with the rest of the groups. Catalase is the main enzyme that scavenges the free radicals produced in the body protecting the cells from peroxidative damage and hence its assay can be counted as an indication of free radical scavenging ability of tissue in question (Vasudevan and Sreekumari, 1998). Since, blood forms the fluid connective tissue circulating throughout the body blood catalase activity may indicate the free radical scavenging ability of the body as a whole or the antioxidant status of the animal in total (Vasudevan and Sreekumari, 1998). In this way, higher enzyme activity in the G-III group (α-tocopherol fed) and G-IV group (combination of ethinylestradiol+levanorgestrel and α-tocopherol) in the present study indicated a better antioxidant status in the birds.

5.4.4.3 Plasma lipid peroxidation

Alpha-tocopherol fed group had the lowest lipid peroxidation value among the groups. It is also intresting to note that both ethinylestradiol+levanorgestrel treated group and birds fed with a combination of ethinylestradiol+levanorgestrel and α-tocopherol also showed lower values compared to control group (table 9 and fig. 13). This indicated that ethinylestradiol+levanorgestrel and α-tocopherol are capable of reducing peroxidation level in plasma. It is now being realized that one of the reasons for majority of toxicities/ disorders is imbalance between amounts of free radicals generated in the body and antioxidants to scavenge and protect the body against their deleterious effects (Halliwell, 1992). Vitamin E deficiency has been reported to be frequently associated with an increased susceptibility to free radical oxidation (Dormandy, 1978). Polyunsaturated fatty acids (PUFA) of membranes are particularly vulnerable to attack by ROS (reactive oxygen species), and ROS can initiate a chain reaction of lipid destruction that destroys the membrane of the cell. Vitamin E can quench peroxidation reactions in membranes and is probably the most important antioxidant located in cell membranes (Putnam and Comben, 1987). The common function that underpins the diverse applications of vitamin E is mainly its

ability to function as an antioxidant in biological systems. Free radicals are neutralized by α-tocopherol before lipid oxidation propagates among highly unsaturated fatty acids in cellular and subcellular membranes (Burton and Ingold, 1989; Hogan *et al.,* 1993;1996). The chromanol ring of α-tocopherol is located among the polar head groups of the phospholipids, and the phytol side chain interacts with the unsaturated fatty acyl chains of the phospholipids through van der Waals interactions in the interior of the membrane (Gomez-Fernandez *et al.,* 1989 and Kagan, 1989). This specific localization of α-tocopherol in the membrane and the molecule's lateral mobility allow it to function very efficiently to protect highly oxidizable polyunsaturated fatty acids from peroxidation by reactive oxygen species produced by adjacent membrane-bound enzymes (Gomez-Fernandez et al., 1989; Liu *et al.,* 1995). Vitamin E is an integral component of all lipid membranes and serves to protect the lipid membranes from attack by reactive oxygen species (Smith *et al.,* 1997). Supplemental vitamin E (250 mg/kg) significantly reduced lipid peroxidation in serum of cold stressed layers (Kucuk *et al.,* 2003). Majority of the literature supports the observations of present study that vitamin E can lower the lipid peroxidation in plasma. However, few authors had doubt on the efficacy of vitamin E to act as peroxidation reducing agents. Siciliano *et al.,* 1997 observed that lipid oxidation measured as TBARS (thiobarbituric acid reacting substances) concentration was not affected by level of vitamin E supplementation in horses and in pigs (Ohene-Adjei *et al.,* 2004) at levels of 100, 200 and 300 mg/kg. Dotan *et al.* (2004) in their review opined that vitamin E failed to show significant correlation with antioxidant enzyme systems while vitamin C did had the effects.

Ethinylestradiol+levanorgestrel was capable of lowering plasma lipid peroxidation at sixth week while it failed at eighth week. The reduction was considerably lesser than that observed in α-tocopherol fed group G-III. There is little literature available which does support the finding of present study at sixth week of age. Esterified estrogen reduced lipid peroxidation products to the extent of greater

than 50% in Cynomealous monkeys challenged with an atherogenic diet (Wagner *et al.*, 1996). Zhu *et al.* (1999) observed that when 17ß-estradiol was added to a system containing LDL and incubated alone and combined in the absence or presence of bovine aortic endothelial cells, placental trophoblast, or macrophages, an antioxidant effect was observed. The lesser action observed in ethinylestradiol+levanorgestrel fed group and birds fed with a combination of ethinylestradiol+levanorgestrel and α-tocopherol in the present study may be attributed to levanorgestrel (progestins), which are known to inhibit antioxidant effect of estrogen (Zhu *et al.*, 1999).

There are also reports in which the estrogen treatment caused an increase in lipid peroxidation. Cho *et al.* (1988) observed that estrogen implants in young male chickens caused a two-fold increase in plasma lipid peroxidation measured by the thiobarbituric acid test.

5.4.5 Effect of ethinylestradiol+levanorgestrel and α-tocopherol on serum minerals

The only significant change observed was the age related increase in Phosphorous concentration and a decrease in Copper at the eighth week of age in all the experiment groups (table 10). Individual treatments failed to elicit any change in all the four minerals (Calcium, Phospherous, Copper and Iron) measured.

With regard to role of estrogens in mineral metabolism the findings in the present study were contradictory to common belief that oestrogen has a beneficial effect on calcium metabolism especially in women. Oestrogen substitution diminishes or stops the loss of bone mass in a significantly more protective mannor in postmenopausal women (Cauley *et al.*, 1995; Writing group., 2002 and Thijssen., 2003) and progestins do not add or subtract much of the protective action of estrogens on the bones (Doren and Samsioe., 2000; Banks, *et al.*, 2001 and Thhijssen., 2003). The results may suggest that estrogens have no role in mineral metabolism in broiler chicken. This may be due to the lower exposure period for treatments.

Findings of the present experiment is congruent with the observations of the earlier study conducted by Arslan *et al.* (2001) where, there was no significant difference in plasma calcium, and phosphorus between control and vitamin E supplemented birds. While, Sahin *et al.* (2002) observed a linear increase in serum calcium and phosphorous concentration with increasing dietary vitamin E up to a level of 250 mg/kg of feed in layers.

5.5 EFFECT OF ETHINYLESTRADIOL+PROGESTERONE AND α-TOCOPHEROL ON TISSUE ENZYME AND ANTIOXIDANT STATUS.

5.5.1 Gama glutamyl transferase (GGT) activity in liver and breast muscle.

Measuring the enzyme status in tissue is a better method for analyzing the status of organs. In the present investigation the activity of GGT was measured in liver as well as breast muscles of broiler chicken (table 11). Ethinylestradiol+levanorgestrel caused significant increase in GGT levels only in the liver of birds of group G-II while there was no such change in breast muscles. The drastic increase in GGT may be due to the cholestatic effect of ethinylestradiol (Davis and Kern., 1976; Rodriquez *et al.,* 1992; Bossard *et al.,* 1993; Puglielli *et al.,* 1994; Simon *et al.,* 1996 and Geier *et al.,* 2003) and also the fact that GGT activity had an increase in conditions of obstructive cholestasis.

5.5.2 Peroxidation level in liver and breast muscle.

Excessive lipid peroxidation can cause deteriorating changes in cellular metabolism of tissues culminating in cellular degradation and ultimately cell death (Winrow *et al.,* 1993). There are a number of compounds which are thought to have peroxidation preventing property. Antioxidants such as α-tocopherol is one among them. In the present investigation α-tocopherol was successful in lowering peroxidation level in tissues (both liver as well as breast muscle) vide table 11. Findings of the present experiment is congruent with the observations of the earlier studies mentioned below.

Dietary supplementation of α-tocopherol increases the α-tocopherol concentration in muscle and its membranous sub-cellular fractions and protects membranal lipids in broiler chicken at 200 mg/kg of feed (Lauridsen *et al.*, 1997), in lambs (Guidera *et al.*, 1997) and in beef (Mitsumoto *et al.*, 1998 and O'Grady *et al.*, 2001). Vitamin E reduced sensory meat rancidity shows the effectiveness of dietary α-tocopheryl acetate supplementation in protecting broiler meat against lipid oxidation (Carreras *et al.*, 2004).

Vitamin E is an integral component of all lipid membranes and serves in protecting lipid membranes from attack by reactive oxygen species (Smith *et al.*, 1997). Lipid oxidation measured as TBARS (thiobarbituric acid reacting substances) concentration was not affected by level of vitamin E supplementation in horses (Siciliano *et al.*, 1997) and in pigs at levels of 100, 200 and 300 mg/kg (Ohene-Adjei *et al.*, 2004).

Increased dietary vitamin E levels of 10 to 20 mg/kg of feed increased the oxidative stability in liver while there was no influence on the oxidative stability of heart and breast muscles (Hsieh *et al.*, 2002).

5.6 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON CRUDE PROTEIN AND ETHER EXTRACT OF BREAST MUSCLES.

Crude protein content did not differ among the various groups (table 12). While all the treatments (ethinylestradiol+levanorgestrel, α-tocopherol and their combination) resulted in significantly lower ether extract value in respective groups when compared to control group. Most of the available literature suggested a positive effect for estrogen treatment on protein content in animals. Implants containing estradiol benzoate and progesterone showed a trend towards greater protein and less fat deposition (Rumsey *et al.*, 1992). According to Rumsey *et al.* (1996) and Elasser *et al.* (1998) Synovex-S (200 mg progestrone and 20 mg estradiol benzoate) implanted steers showed greater protein and water gains. Estaradiol was found to

reduce fat around organelles (Enright *et al.*, 1990) and rib section (Lee *et al.*, 1990) in cattle when administered as ear implants. Field *et al.* (1990) found no significant effect on fat deposition in lambs implanted with estradiol. The probable reason for the lower ether extract value in ethinylestradiol+levanorgestrel fed groups G-II and G-IV (ethinylestradiol+levanorgestrel and α-tocopherol) may be due to inhibition of lipogenesis (Green *et al.*, 1992).

5.7 EFFECT OF ETHINYLESTRADIOL+LEVANORGESTREL AND α-TOCOPHEROL ON HISTOMORPHOLOGY OF LIVER, ADRENAL AND BREAST MUSCLES

Only liver sections showed any significant changes (plates 1 to 8). Adrenal and breast muscle structure did not show any variation among the groups (plate 9 to 16). Livers of estrogen exposed fish have been reported to be enlarged, accumulated with amorphous eosinophilic material or simply resembled the liver of vitellogenic female with increased basophilia and enlarged nuclei (Wester and Canton. 1986, Hamzaki *et al.*, 1987, Herman and Kinacid. 1988, Gimeno *et al.*, 1998a,b; Dreze, *et al.*, 2000. Lange *et al.*, 2001, Metcalfe *et al.*, 2001, Zillioux *et al.*, 2001 and Weber *et al.*, 2003). In this instance no such change was observed. The liver cells had significantly lesser fat accumulation compared to all the other groups which was consistent with the finding that ethinylestradiol+levanorgestrel lowered the fat levels in plasma. The liver in Group I, Group III and Group IV had a condition called fatty change due to the excess accumulation of fat in hepatocytes. According to Hsieh *et al.* (2002) monounsaturated fatty acid, saturated fatty acid, and polyunsaturated fatty acid content in heart, liver, and breast muscle were not affected by dietary vitamin E level (0, 10 or 30 mg/kg). Soler –Velasquez *et al.* (1998) also didn't observe any effect of dietary vitamin E on fatty acid composition of liver in finishing swine.

Summary

6. SUMMARY

Although research/investigations on the effect of supplementation of various steroids as ethinylestradiol+levanorgestrelic preparations and α-tocopherol on the metabolism of humans and various animals have been done, meager attempts have been done in case of avian species like broiler chicken. The experiment was conducted in commercial broiler chicken of Vencob strain to study the effect of dietary supplementation of steroid hormones (combination of ethinylestradiol and levanorgestrel) and α-tocopherol.

Thirty two, day old chicks of Vencob strain were procured, divided into four groups containing eight chicks in each group as G-I (control), G-II, G-III, and G-IV (treatments) and reared under identical management conditions for a period of four weeks. The standard broiler ration (starter and finisher) formulated as per Bureau of Indian Standards specifications (BIS, 1992) and proximate principle was estimated as per Association of Official Analytical Chemists (AOAC, 1990). From fourth to eighth week of age, birds were fed with the ration as G-I (control) fed with standard broiler finisher ration, G-II fed with Standard broiler finisher ration + ethinylestradiol and levanorgestrel incorporated @ 66.3 mg & 331.5 mg respectively per 100 kg of feed, G-III fed with Standard broiler finisher ration + α-tocopherol @ 25 g per 100 kg of feed and G-IV fed with Standard broiler ration + α-tocopherol @ 25 g and a combination of ethinylestradiol and levanorgestrel @ 66.3 mg & 331.5 mg respectively per 100 kg of feed.

The birds were daily watched for their health status. The body weight of birds in each group was recorded initially, thereafter at weekly intervals till eighth week and weight gain was calculated. Blood samples (5 ml each) were collected with and without anticoagulant from wing vein at fortnightly intervals from four weeks onwards and at the end of experiment for the analysis of hematological, biochemical, enzymatic, antioxidant status and mineral estimations. Blood samples collected with

anticoagulant (heparin 20 IU/ml of blood) were subjected to the estimation of various haematological parameters. Plasma was separated from blood samples for the various investigations. Tissue samples were collected (liver, muscle and adrenal) for analysis of enzymes, tissue peroxidation level, crude protein and ether extract. Histological sections of liver, adrenal and muscle were prepared and examined. Statistical analysis was done using appropriate tools.

Ethinylestradiol+levanorgestrel supplementation significantly ($P<0.05$) lowered total erythrocyte count (TEC), haemoglobin (Hb) concentration, volume of packed red cells (VPRC and plasma total cholesterol concentration while α-tocopherol did not bring such effect. In the present study ethinylestradiol+levanorgestrel and α-tocopherol either alone or in combination significantly increased the plasma high density lipoprotein (HDL) levels in birds from sixth to eighth week of age. There was also age related increase in all the treatment groups (G-II, G-III and G-IV). There was significant ($P<0.05$) lowering of plasma concentration of LDL in the ethinylestradiol+levanorgestrel fed G-II group. There was considerable lowering of the plasma concentration of VLDL in the ethinylestradiol+levanorgestrel (G-II), α-tocopherol (G-III) and ethinylestradiol+levanorgestrel and α-tocopherol (G-IV) fed groups throughout the experiment. Ethinylestradiol+levanorgestrel and α-tocopherol alone or in combination caused reduction in the levels of plasma VLDL and plasma triglycerides in broiler chicken. Ethinylestradiol+levanorgestrel caused significant reduction in total lipid content especially in G-II while α-tocopherol fed group G-III had significantly ($P<0.05$) higher concentration of plasma total lipids at sixth week of age. Among the periods, the total plasma lipid concentration was significantly ($P<0.05$) higher in birds of α-tocopherol fed group G-III at sixth week when compared to fourth week. Both G-I and G-III group of birds recorded reduction in their plasma concentration of total lipids by eighth week compared to sixth week.

Whereas in ethinylestradiol+levanorgestrel treated group (G-II) there was significant ($P<0.05$) decrease in total lipids with the advancing age.

Plasma GGT activity of the birds of ethinylestradiol+levanorgestrel fed group (G-II) was the highest and the birds of α-tocopherol supplemented group (G-III) had the lowest value. Among the periods there was significant influence ($P<0.05$) of age on plasma GGT concentration of birds of ethinylestradiol+levanorgestrel fed group (G-II), which showed drastic increase in GGT values with age (highest at sixth week with slight reduction at eighth week). The plasma AST activity also showed similar trend as that of GGT activity with drastic increase ($P<0.05$) in values at sixth and eighth week of age in the ethinylestradiol+levanorgestrel treated group (G-II).

Antioxidant status of animals is usually assayed by estimating the activity of free radical scavenging enzymes like superoxide dismutase (SOD), catalase and also through measuring the peroxidation levels. In the present investigation assessment of antioxidant status of the chicken has been confined to the activity of SOD and catalase as well as lipid peroxidation levels. Both ethinylestradiol+levanorgestrel and α-tocopherol had significant effect on the plasma SOD activity at sixth week of age when compared with other groups, while the declining trend in activity was observed in all groups from fourth to eighth week. In the present study α-tocopherol alone or when combined with ethinylestradiol+levanorgestrel resulted in a significant increase in the catalse activity. This was observed only at sixth week of age. Among the periods there was significant influence ($P<0.05$) of age in plasma catalase activity of birds in all the four groups with an increase in activity by the sixth week. The increase was more prominent in α-tocopherol fed groups (G-III and G-IV). The higher enzyme activity in the G-III group (α-tocopherol fed) and G-IV group in the present study indicated a better antioxidant status in these birds. Birds of α-tocopherol fed group had the lowest lipid peroxidation value among the groups. It is also interesting to note that both ethinylestradiol+levanorgestrel treated group and birds fed with ethinylestradiol+levanorgestrel and α-tocopherol also showed lower

values compared to control group, which proved both are capable of reducing peroxidation level in the body.

An age related increase was observed in the serum concentration of Phosphorous and a decrease in Copper level at the eighth week of age in all the experiment groups. Individual treatments failed to elicit any change. The findings in the present study were contradictory to common belief that oestrogen has a beneficial effect on the calcium metabolism.

In the present investigation the activity of GGT was measured in liver as well as breast muscles of broiler chicken. Ethinylestradiol+levanorgestrel caused significant increase in GGT levels only in the liver of G-II birds while there was no such change in breast muscles. Excessive lipid peroxidation can cause deteriorating changes in cellular metabolism of tissues culminating in cellular degradation and ultimately cell death. In the present investigation α-tocopherol was successful in lowering peroxidation in tissues (both liver as well as breast muscle).

Liver from birds of ethinylestradiol+levanorgestrel treated birds had significantly lesser fat accumulation compared to all the other groups. The liver in G-I, G-III and G-IV had a condition called fatty change due to the excess accumulation of fat in hepatocytes.

The body weight of birds in various groups did not differ significantly through out the experimental period. Alpha-tocopherol alone or in combination with ethinylestradiol+levanorgestrel resulted in significant improvement in average daily gain (ADG) in broiler birds. Ethinylestradiol+levanorgestrel and α-tocopherol alone or in combination failed to influence the feed efficiency in the experimental birds. Dressing percentage was significantly improved in the broiler chicken when dietary supplementation of ethinylestradiol+levanorgestrel and α-tocopherol alone were given.

Dietary supplementation of ethinylestradiol+levanorgestrel and α-tocopherol failed to elicit much anticipated effect in growth and other performance indices. In

general ethinylestradiol+levanorgestrel exposure lead to hypolipedemic state with reduction in the level of plasma total cholesterol, LDL and VLDL cholesterol. This is a beneficial effect in that most of these parameters are other wise responsible for complications such as atherosclerosis. Alpha-tocopherol has been proven once again their protective role against oxidative damage of cells. Ethinylestradiol was also found to impart protection against oxidative damage. However, ethinylestradiol+levanorgestrel resulted in damage to the biliary system resulting in elevated levels of enzymes such as the concentration of GGT and AST. Dietary supplementation of ethinylestradiol+levanorgestrel and α-tocopherol were found to be not beneficial to the expected level and they only added to the already increasing production cost in the poultry industry. The steroid preparations for dietary supplementation are unavailable in the Indian market too and they are highly costly. Hence, it is concluded that they are not incorporated as a growth promoter in broiler ration.

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PHYSIOLOGICAL EVALUATION OF DIETARY SUPPLEMENTATION OF STEROID HORMONES AND ALPHA-TOCOPHEROL IN BROILER CHICKEN

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ABSTRACT

Thirty two day old broiler chicks of Vencob strain were divided into four groups containing eight chicks in each group as G I (control) G II G III and G IV (treatments) and reared under identical management conditions for a period of four weeks with standard broiler ration as per BIS (1992) From fourth to eighth week of age birds of group G I (control) fed with Standard broiler finisher ration G II fed with Standard broiler finisher ration + Ethinylestradiol and Levanorgestrel incorporated @ 66 3 mg & 331 5 mg respectively per 100 kg of feed G III fed with Standard broiler finisher ration + dl α tocopherol @ 25 g per 100 kg of feed and G IV fed with Standard broiler finisher ration + dl α tocopherol @ 25 g and a combination of Ethinylestradiol and Levanorgestrel @ 66 3 mg & 331 5 mg respectively per 100 kg of feed

Birds were regularly monitored for clinical health status feed intake and individual weight (weekly intervals) Blood samples (5 ml each) were collected with and without anticoagulant at fortnightly intervals from fourth week to the end of experiment and analysed hematological parameters plasma protein profile lipid profile enzymatic activity such as gama glutamyltransferase (GGT) aspartate aminotransferase(AST) superoxide desmutase (SOD) catalase and lipid peroxidation and serum concentration of calcium phosphorus copper and iron were also analysed Histomorphology of liver adrenal and breast muscles were conducted Liver and breast muscles were analysed for enzymes as GGT tissue peroxidation and crude protein and ether extract Data were analysed statistically with appropriate tools

Results obtained showed that dietary supplementation of ethinylestradiol+levanorgestrel and dl α tocopherol failed to elicit any noticeable influence on the growth and feed efficiency and mineral status (serum calcium phosphorous copper and iron levels) m the experimental birds Ethmylestradiol+levanorgestrel supplementation significantly lowered ($P<0.05$) total erythrocyte count (TEC) haemoglobin (Hb) and volume of packed red cells (VPRC)

Ethinylestradiol+levanorgestrel supplementation significantly (P<0 05) lowered the plasma concentration of total lipids total cholesterol and LDL (birds of G II group) while dl α tocopherol did not bring such an effect Ethinylestradiol+levanorgestrel and dl α tocopherol either alone or in combinat on significantly increased the plasma high density lipoprotein (HDL) levels in birds from sixth to eighth week of age Ethinylestradiol+levanorgestrel and dl α tocopherol alone or in combination caused reduct on in plasma VLDL and triglyceride values There was a lowei level of ether extract in muscles of all the treatment groups compared with control Liver from birds of ethinylestradiol+levanorgestrel treated biids (G II) had lowei fat accumulation in hepatocytes

Ethinylestradiol+levanorgestiel fed group G II (P<0 05) had the highest and the birds of dl α tocopherol supplemented group G III had the lowest plasma GGT and AST act vity at sixth and eighth week of age These effects might be due to the effects of ethinylestradiol+levanorgestrel on the biliary system resulting in ai levated enzyme activity in plasma Similarly ethinylestrad ol+levanorgestrel caused ignificant increase in GGT levels only in the liver of G II birds while there was no uch change in breast muscles Dl α tocopherol alone (G III) or when combined with thinylestradiol+levanoigestrel (G IV) caused a significant increase (P<0 05) n atalse activ ty while ethinylestrad ol+levanorgestrel and dl α tocopherol alo ie (G II and G III espectively) or in combii ation (G IV) also showed loweied l pid eroxidat on *which* ii dicated a better antioxidant status Predominant effect of thinylestradiol+lev anorgestrel was observed on the plasma lip d prof le (reduction in he level of plasma total cholesterol LDL VLDL cholesterol) while α tocopherol howed its antioxidant properties However ethinylestradiol +levanorgestrel caused he damage to the biliary system resulting in elevated levels of enzymes such as GGT and AST Dietary supplementation of ethinylestradiol+levanorgestrel and dl α ocopherol failed to elicit much anticipated effect on growth and other performance nd ces