EFFECT OF BLUE GREEN ALGA (*Spirulina platensis*) ON HAEMATOLOGICAL, BIOCHEMICAL AND FERTILITY PARAMETERS OF EGG TYPE MALE CHICKEN

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Thesis submitted in partial fulfillment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled **"EFFECT OF BLUE GREEN ALGA (*Spirulina platensis*) ON HAEMATOLOGICAL, BIOCHEMICAL AND FERTILITY PARAMETERS OF EGG TYPE MALE CHICKEN"** is a bonafide record of research work done by me during the course of research and that the 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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CERTIFICATE

Certified that the thesis entitled **"EFFECT OF BLUE GREEN ALGA (*Spirulina platensis*) ON HAEMATOLOGICAL, BIOCHEMICAL AND FERTILITY PARAMETERS OF EGG TYPE MALE CHICKEN"** is a record of research work done independently by **Dr. Sethu.C. Nair** 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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We, the undersigned members of the Advisory Committee of **Dr. Sethu.C. Nair,** a candidate for the degree of Master of Veterinary Science in Physiology, agree that the thesis entitled **"EFFECT OF BLUE GREEN ALGA (*Spirulina platensis*) ON HAEMATOLOGICAL, BIOCHEMICAL AND FERTILITY PARAMETERS OF EGG TYPE MALE CHICKEN"** may be submitted by Dr. Sethu.C. Nair in partial fulfilment of the requirement for the degree.

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Introduction

1. INTRODUCTION

Indian poultry industry has grown rapidly at a rate of 15 to 20% during the last two decades and is now a Rs.65 billion mega-industry providing employment to 1.5 million people. With an annual output of 30, 000 million eggs and 1, 000 million broiler birds yielding 0.5 million tonnes of poultry meat, India ranks fourth largest producer of eggs and eighth largest producer of poultry broiler in the world (Banerjee, 2002). The accomplishment takes its attributes to scientific breeding and management ordained through tenacious research.

Breeding, by way of streamlining the innate drive proffered by the genetic fabric of the stock, stood the first line of impulsion in achieving enhanced livestock/poultry production. The second line was in providing improved nutrition and management. However, livestock/poultry farming has to meet the new demands of cost-effective means of production adoptable to the farmers/entrepreneurs and provision for safe and wholesome products to the consumers, in order to sustain in the new economy with an open global market. Towards this goal, nutritional manipulations have been highly instrumental, not surprising since nutrition forms the single largest external factor affecting performance of the stock and accounting lion share of the input cost. Moreover, dietetic methods are very easy to adopt and non-invasive to practice. Especially in the wake of set backs from potential residual hazards of anabolic steroids used as growth promoting feed additives, research endeavours round the globe are now more inclined towards more innocuous alternatives, emphatically organic candidates, for being safe to the subject, consumers and environment.

Cyanobacteria (blue green algae) are now well noted with a wide market presence owing to their anabolic and therapeutic effects in human health. Livestock and poultry industries have picked the string entailing enormous scope for research in this area. Some of the studies exploring the nutritional and

therapeutic potentials of these algae have emerged with promising results in animal production too.

Spirulina, a unicellular filamentous blue-green alga, is generally regarded as a good source of nutrients by virtue of its high protein, vitamins and mineral contents. Protein from spirulina contains 18 amino acids, major ones being lysine, arginine, threonine, methionine and phenylalanine. High levels of arginine is normally believed to be insulinogenic, indirectly through stimulation of growth hormone secretion (Ganong, 1996). The absence of thick cell wall (typical of plant and algal cells) may contribute to the high digestibility of spirulina protein (Mepham, 1997).

It is noteworthy that many species of cyanobacteria boast exuberant carotenoid content and hence serve as a relatively inexpensive source of vitamin A (Mepham, 1997). In turn, the role of vitamin A in reproduction is important in the sense that a deficiency of vitamin A in the diet ultimately causes complete failure of reproduction (Hafez and Hafez, 1993). Similarly, there is also evidence that a prolonged vitamin E deficiency results in sterility in the male and reproductive failure in the female, and male sterility may become permanent through degenerative changes in the testes (Mc Donald, 1990). As per reports spirulina is a rich source of vitamin E too (Yoshida and Hoshii, 1990). In addition, macro and micro mineral content of these blue green algae (Ross and Dominy, 1990) is also tempting to anticipate accentuation of fertility parameters.

However, majority of earlier studies involving spirulina are relating to human health whereas experimentation in farm animals is just gaining momentum. Growth stimulating and therapeutic effects of spirulina are the current priorities in livestock and poultry production. Whereas feeding trials in broiler type chicken with spirulina supplementation have largely been successful in bringing forth improved growth and immune status, there are meagre reports addressing the influence of this alga in egg type chicken. All the more, studies on males of layer type birds veritably lack in published literature.

In layer type chicken, akin to growth related parameters fertility aspects also assume considerable importance unlike in broiler birds since the latter ones are slaughtered very early in their life. Economically, fertility of males in a breeding flock is thought to be of greater importance than that of the females. In fact, males of low or questionable fertility may go undetected many a times because most of the time they are sexually aggressive and appear healthy and normal within a flock. So, fertility evaluation will be worth enough only once the semen and testicular characteristics are looked upon. Furthermore, growth enhancing and fertility augmenting effects of extraneous agents can well be apparent during the growing phase of the subjects. The currently proposed study is thus expected to initiate meaningful exploration of growth promoting and fertility augmenting aspects of *Spirulina platensis*.

Hence a study involving dietary supplementation of *Spirulina platensis* in growing egg type male chicken with the following objectives has been contemplated:

1. To monitor the changes in certain haematological and biochemical parameters.

2. To assess the free radical scavenging effects, if any.

3. To find out the fertility augmenting effects, if any

Review of Literature

2. REVIEW OF LITERATURE

Spirulina, the blue green alga, serves as a food supplement recommended for both animals and humans. Being a rich source of many nutrients like proteins, vitamins and many essential amino acids spirulina is nicknamed as "super food". Recent studies have also revealed many therapeutic properties of this alga.

Blinkova *et al.* (2001) reviewed the biological activity of spirulina. They opined that *Spirulina platensis* (SP) have diverse activities due to high content of proteins, indispensable amino acids, vitamins, beta-carotene and other pigments, minerals, indispensable fatty acids and polysaccharides. *Spirulina platensis* has been found suitable for use as a bioactive additive. It enhances resistance in humans, mammals, chickens and fishes to infections. The immunostimulating effect of spirulina could be by way of its action in haemopoiesis, stimulating antibody and cytokine production and activating T & B lymphocytes. *Spirulina platensis* sulfolipids have proved to be effective against human immunodeficiency virus (HIV). Preparations obtained from SP biomass have also been found active against herpes virus, cytomegalo virus, influenza virus, etc. Besides SP extracts were capable of inhibiting carcinogenesis. Preparations of spirulina are believed to contribute to the destruction of *Candida albicans* and preservation of the resident intestinal microflora, especially lactic acid bacilli and bifidobacteria. Such interactions of SP with microorganisms offer good promise for using this micro-alga as a component of culture media.

2.1 COMPOSITION AND NUTRITIVE VALUE OF SPIRULINA

Clement (1975) has reported that spirulina is rich in protein. The alga also contains nucleic acids (about 4% of the dry matter) and saturated C-16 and ethylenic C-18 fatty acids, gamma linoleic acid being the characteristic fatty acid of *S. plantesis.* The unsaponifiable content is made particularly of sterols such as cholesterol, beta-sitosterol and triterpenic alcohols such as alpha-amyrin.

Annapurna *et al.* (1991) conducted experiments to assess spirulina as a source of vitamin A in rats. In one experiment, rats were given spirulina as the sole source of vitamin A whereas the controls were fed synthetic vitamin A. Liver content of vitamin A in spirulina-fed rats of both sexes was found to be significantly higher than that of the control rats. In another experiment, availability of carotene from the solvent extract of spirulina (vitamin A value) as judged by the levels of vitamin A and carotene in plasma and liver in male rats were compared with those of synthetic beta-carotene or vitamin A. The level of vitamin A in the liver and plasma of Spirulina carotene-fed rats was much higher than those of the controls.

Kapoor and Mehta (2000) compared bioavailability of iron from spirulina with that from whole egg, whole wheat, and standard ferrous sulphate. They observed that even when the absorption of iron from spirulina was significantly lower than that of ferrous sulphate and whole egg, haemoglobin regeneration efficiency of spirulina and whole egg was similar and was significantly higher than that of whole wheat.

Mendzhul *et al.* (2000) studied the activity and physico-chemical properties of tricarboxylic acid (TCA) cycle enzymes and associated enzymes such as isocitrate lyase and glutamate dehydrogenase of *Spirulina platensis*. High activities of most of the enzymes studied except alpha-ketoglutarate dehydrogenase (alpha-KGDH) and succinate dehydrogenase have been found. Results reflected spirulina's ability to synthesize organic substances intensively, especially proteins. Absence of alpha-KGDH activity indicated that TCA cycle of spirulina mainly performs the biosynthetic function with limited energy generation.

2.1.1 Immunomodulating Properties

Baojiang *et al.* (1994) opined that polysaccharides of spirulina, at a dosage of 150 to 300 mg/kg body weight, by injection or orally, increased the

phagocyte percentage and phagocytic index of abdominal macrophages, the percentage of T-lymphocytes and haemolysin content in the peripheral blood of mouse. The mechanism could be related to polysaccharides, which enhanced reproductive ability of marrow cells, growth of thymus and spleen and biosynthesis of serum protein. These results demonstrated that spirulina polysaccharides could improve both nonspecific cellular immunity and specific humoral immunity.

Hayashi *et al.* (1994) also suggested that Spirulina enhanced the immune response, particularly the primary response, by stimulating macrophage functions, phagocytosis, and interleukin-1 (IL-1) production. They observed that mice fed on a *Spirulina platensis* diet showed increased numbers of spleenic antibody-producing cells against sheep red blood cells in the primary immune response. However, immunoglobulin G (IgG) production in the secondary immune response was hardly affected. The percentage of phagocytic cells in peritoneal macrophages from the mice fed on *Spirulina platensis* diet was significantly increased. Addition of a hot-water extract of *Spirulina platensis* (SHW) to an *in vitro* culture of spleen cells increased proliferation of these cells, whereas culture of thymus cells was scarcely affected. The Spirulina extract also significantly enhanced IL-1 production from peritoneal macrophages.

Qureshi and Ali (1996) studied the influence of spirulina extract on macrophage phagocytic function in cats. Through bronchoalveolar lavage, macrophages isolated from cats were cultured on glass cover slips. Macrophages were then exposed to a water-soluble extract of *Spirulina platensis* in concentration range of 0 to 60 µg/ml for two hours. Spirulina-extract exposure did not cause significant macrophage cytotoxicity over untreated control cultures. Macrophage monolayers from treated and control cultures were incubated with sheep red blood cells (SRBC) and viable *Escherichia coli*. The percentages of phagocytic macrophages for both of these particulate antigens were higher (a two-fold increase in SRBC phagocytosis and over 10% increase in *Escherichia coli* uptake) in cultures treated with various concentrations of spirulina-extract.

However, the numbers of either type of particles internalized by phagocytic macrophage were not different between the control and treated cultures.

Yang *et al.* (1997) investigated the effects of the powders of *Spirulina platensis* (SPP) on anaphylactic reactions. *Spirulina platensis* powder inhibited the compound 48/80 induced anaphylactic shock with doses of 0.5, and 1.0 mg/g body weight. *Spirulina platensis* powder significantly inhibited serum histamine levels induced by compound 48/80 in rats. Spirulina platensis powder at a dose rate of 0.5 mg/g body weight inhibited 68.7% passive cutaneous anaphylaxis activated by anti-dinitrophenyl (DNP) IgE. *Spirulina platensis* powder dose-dependently inhibited the histamine release from the rat peritoneal mast cells (RPMC) by compound 48/80. Moreover, SPP had a significant effect on anti-DNP IgE-induced histamine release or tumor necrosis factor-alpha (TNF-α) production from RPMC. These results suggested that SPP may contain compounds with actions that inhibit mast cell degranulation in the rat.

Hayashi *et al.* (1998) studied the antibody production in mice as possible evidence of the protective effects of *Spirulina platensis* towards food allergy and microbial infection. An increase of IgE antibody level in the serum was observed in mice that were orally immunised with crude shrimp extract as an antigen. The antibody level, however, was not further enhanced by treatment with spirulina extract. IgG1 antibody, which was increased by antigen administration, was further enhanced by spirulina extract. It was noted that the IgA antibody level in the intestinal contents was significantly enhanced by treatment with spirulina extract concurrently ingested with shrimp antigen, in comparison with that of the group treated with shrimp antigen alone. An enhancement of IgA antibody production by spirulina extract was also observed in culture supernatant of lymphoid cells, especially in the spleen and mesenteric lymph node from mice treated with spirulina extract for four weeks before antigen stimulation. It was concluded that spirulina neither induced nor enhanced allergic reaction such as food allergy dependent on an IgE antibody. On the contrary, before antigen

stimulation and when ingested concurrently with antigen, spirulina significantly enhanced the IgA antibody level to protect against allergic reaction.

The effect of spirulina on mast cell-mediated immediate-type of allergic reactions was investigated by Kim *et al.* (1998). Spirulina dose-dependently inhibited the systemic allergic reaction induced by compound 48/80 in rats at the dose rate of 100-1000 μg/g body weight and also significantly inhibited local allergic reaction activated by DNP IgE. When rats were pretreated with spirulina at a concentration ranging from 0.01 to 1000 μg/g body weight, the serum histamine levels were reduced in a dose-dependent manner. Spirulina (0.001 to 10 μg /mL) dose-dependently inhibited histamine release from RPMC activated by compound 48/80 or anti-DNP IgE. When spirulina (10 μg /mL) was added, the level of cyclic AMP in RPMC transiently and significantly increased about 70 fold at 10 seconds compared with that of control cells. Moreover, it had a significant inhibitory effect on anti-DNP IgE-induced TNF-α production.

Pugh *et. al.* (2001) identified three new high molecular weight polysaccharide preparations isolated from food-grade microalgae that are potent activators of human monocytes/macrophages and they named "Immulina" for the one that was isolated from *Spirulina platensis*. These polysaccharides are structurally complex and have estimated molecular weights above 10^7 daltons. All three polysaccharides are highly water-soluble and comprise between 0.5 % and 2.0 % of microalgal dry weight. Each polysaccharide substantially increased mRNA levels of interleukin-1beta (IL-1beta) and TNF-α. These polysaccharides are between 100 and 1000 times more active for *in vitro* monocyte activation than polysaccharide preparations that are currently used clinically for cancer immunotherapy.

2.1.2. Hypolipidaemic Properties

The effects of spirulina on lipoprotein lipase activity and hepatic triglyceride lipase activity in plasma were studied in fructose-induced

hyperlipidemic rats by Iwata (1990). Male Wistar rats aged 3 weeks (body weight, 54g) were fed on the high-fructose diet (68%) alone or the high-fructose diets containing spirulina at levels of 5%, 10%, and 15% for 4 weeks. The dietary hyperlipidemia caused by the high-fructose diet was improved by spirulina feeding, accompanied by a significant increase in the lipoprotein lipase activity in post-heparin plasma. Torres-Duran *et al.* (1998) assessed the capacity of *Spirulina maxima* to prevent fatty liver development induced in rats by an intraperitoneal single dose (1 ml/kg) of carbon tetrachloride. Liver and serum lipids were quantified two or four days after treatment with this agent. Liver lipid concentration did not differ in rats fed on a purified diet with or without spirulina. However, after carbon tetrachloride treatment, liver triacylglycerols were significantly lower in rats fed on a diet with spirulina (5%) than in rats without spirulina in their diet ($P < 0.05$). Furthermore, the increased liver cholesterol values, induced by carbon tetrachloride treatment, were not observed in rats that received spirulina. These results support the potential hepatoprotective role of spirulina.

Parikh *et al.* (2001) reported the role of spirulina in the control of hyperglycemia and lipidemia in type 2 diabetes mellitus and suggested the beneficial effect of spirulina supplementation in controlling blood glucose levels and in improving the lipid profile of subjects with type 2 diabetes mellitus.

Rodriguez-Hernandez *et al.* (2001) opined that *Spirulina maxima* prevented fatty liver formation in male and female mice with experimental diabetes. *Spirulina maxima* (SM) was orally administered at 5% level for four weeks to diabetic mice, starting one week after a single dose of alloxan, 250 mg/Kg body weight. The main action of SM was on triacylglycerol levels in serum and liver. There was also a moderate hypoglycemia in male mice. A decrease in the percentage of HDL in diabetic mice that was reverted by the SM administration was also observed. The sum of LDL + VLDL percentages was also partially normalised in diabetic animals by the SM administration. An additional observation was the lower incidence of adherences between the liver

and the intestine loops in the diabetic mice treated with SM compared with those without SM. Male and female mice showed differences to diabetes susceptibility and response to SM, the female being more resistant to diabetes induction by alloxan and more responsive to the beneficial effects of SM.

Samuels *et al.* (2002) studied the hypocholesterolemic effect of spirulina in patients with hyperlipidemic nephrotic syndrome and concluded that spray-dried spirulina capsules, rich in antioxidants, gamma linolenic acid (GLA), amino acids, and fatty acids, helped in reducing the increased levels of lipids in patients with hyperlipidemic nephrotic syndrome.

2.1.3. Anti-cancer Properties

An extract of spirulina alga was shown to prevent tumor development in hamster buccal pouch when a 0.1% solution of 7, 12-dimethylbenz-a-anthracene (DMBA) in mineral oil was applied topically three times weekly for 28 weeks (Schwartz *et al.,* 1988). At the end of the experiment, all untreated controls presented gross tumors of the right buccal pouch. Animals fed beta-carotene demonstrated a small but statistically significant reduction in tumor number and size. Animals fed canthaxanthin exhibited a notably and statistically significant reduction in tumor number and size compared with controls. Interestingly, the spirulina administered animals presented a complete absence of gross tumors. However, microscopic sections of the buccal pouch in the latter group showed localized areas of dysplasia and early carcinoma *in situ* undergoing destruction.

Dasgupta *et al.* (2001) studied the effect of spirulina supplementation at 250 and 500 mg/kg body weight on drug metabolising phase I and phase II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase and lipid peroxidation in the liver of seven week old Swiss Albino mice. The implications of these biochemical alterations have been further evaluated adopting the protocol of benzo-a-pyrene induced forestomach and 7, 12 dimethylbenz-a-anthracene (DMBA) initiated and croton oil promoted skin

papillomagenesis. Spirulina induced only the phase II enzyme activities associated mainly with carcinogen detoxification. The glutathione S-transferase specific activities were induced in hepatic and all the extrahepatic organs like lung, kidney and forestomach by spirulina pre-treatment. This chemopreventive response was quantitated by the average number of papillomas per effective mouse (tumor burden) as well as percentage of tumor bearing animals. There was a significant inhibition of tumor burden as well as tumor incidence in both the tumor model systems studied. In the skin tumor studies tumor burden was reduced from 4.86 to 1.20 and 1.15 by the low and high dose treatments respectively. In stomach tumor studies tumor burden was 2.05 and 1.73 by the low and high doses of spirulina treatment as against 3.73 of the control group.

Zhang *et al.* (2001) studied chemo and radio-protective effects of polysaccharide of *Spirulina platensis* (PSP) on hemopoietic system of mice and dogs. They observed effect of PSP on the hematopoietic system of mouse and dogs, which were damaged by injection of cyclophosphamide (CTX) and ^{60}Co-gamma irradiation. Cyclophosphamide and ^{60}Co-gamma ray were used to induce bone marrow damage, and the experimental animals were injected with different dose of PSP *in vivo*. After 12 days and 21days of administration, the whole blood cells and nucleated cells in bone marrow were measured, and the DNA in bone marrow was inspected by UV-spectrophotometer. Cyclophosphamide and ^{60}Co-gamma irradiation induced hemopoietic system damage in mice and dogs, respectively. Polysaccharide of *Spirulina platensis* at a dose rate of 30 mg/kg body weight and at 60 mg/kg body weight increased the level of the white cells in blood and nucleated cells and DNA in bone marrow in mice but had no effects on red blood cells and haemoglobin. The PSP @ 12 mg/kg of body weight increased the level of red cells, white cells, and haemoglobin in blood and nucleated cells in bone marrow in dogs ($P<0.01$), and the effects of PSP 60 mg/kg of body weight were better.

2.1.4 Antiviral Properties

Hayashi *et al.* (1996) reported that bioactivity-directed fractionation of a hot water extract from a blue-green-alga *Spirulina platensis* led to the isolation of a novel sulfated polysaccharide named Calcium Spirulan (Ca-SP) as an antiviral principle. This polysaccharide was composed of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, sulfate and calcium. The anti-human immunodeficiency virus type 1 (HIV-1) and anti-herpes simplex virus type 1 (HSV-1) activities of Ca-SP were compared with those of dextran sulfate (DS) as a representative sulfated polysaccharide. Data indicated that Ca-SP was a potent antiviral agent against both HIV-1 and HSV-1. Thus, Ca-SP could be a useful agent for an anti-HIV therapeutic drug that might overcome the disadvantages observed in many sulfated polysaccharides. Ayehunie *et al.* (1998) opined that an aqueous extract of *Spirulina platensis* inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC), and Langerhans cells (LC). They concluded that aqueous *Spirulina platensis* extracts contain antiretroviral activity that may be of potential clinical interest.

Hernandez-Corona *et al.* (2002) evaluated the antiviral activity found in a hot water extract (HWE) of a commercial preparation of spirulina, studied by a microplate inhibition assay, using several viruses. The HWE inhibited the infection of herpes simplex virus type 2 (HSV-2), pseudorabies virus, human cytomegalovirus, and HSV-1, and the 50% effective inhibition doses (ED_{50}) were 0.069, 0.103, 0.142, and 0.333 mg/ml of HWE for each virus, respectively. For adenovirus the inhibition was less than 20%, and no inhibition was found for measles virus at concentrations of 2 mg/ml of the HWE. The highest antiviral activity was for HSV-2.It was also reported that the antiviral activity was not due to a viricidal effect. Herpes virus infection was inhibited at the initial events (adsorption and penetration) of the viral cycle.

2.1.5 Anti-oxidant Properties

Miranda *et al.* (1998) determined the antioxidant activity of a methanolic extract of spirulina *in vitro* and *in vivo* . The *in vitro* antioxidant capacity was tested on a brain homogenate incubated at 37°C and the inhibitory concentration (IC_{50} -concentration which causes a 50% reduction of oxidation) was 0.18 mg/ml. The *in vivo* antioxidant capacity was evaluated in plasma and liver of rats receiving 5 mg/day of spirulina extract for two and seven weeks. The production of oxidised compounds in liver after two hours of incubation at 37°C was measured in terms of thiobarbituric acid reactant substances (TBARS) in control and experimental groups and the antioxidant capacity of plasma was significantly higher for the experimental group when compared to the controls. Data from liver spontaneous peroxidation studies were not significantly different between groups. Bhat and Madyastha (2000) opined that C-Phycocyanin (from *Spirulina platensis*) effectively inhibited Carbon tetrachloride induced lipid peroxidation in rat liver *in vivo*. Phycocyanin, a potent peroxyl radical scavenger significantly inhibited peroxyl radical-induced lipid peroxidation in rat liver microsomes and the inhibition was concentration dependent. These studies have demonstrated that phycocyanin is a potent peroxyl radical scavenger

Upasani *et al.* (2001) studied the effect of Lead with and without vitamin E, C, or spirulina on malondialdehyde, conjugated dienes and hydroperoxides in rats. Lead (100 ppm) was given in doubly deionised water for 30 days to one group of rats. The other groups received lead along with exogenous antioxidants like vitamin E (50 IU/kg), vitamin C (ascorbic acid; 800 mg/kg) or spirulina (1500 mg/kg) in food for a similar period. Levels of lipid peroxidation products such as malondialdehyde, conjugated diene and hydroperoxide were measured in liver, lung and kidney of treated rats. In Lead treated animals there was a significant increase in the levels of these lipid peroxidative products. Administration of exogenous antioxidants in animals reduced the levels of malondialdehyde, conjugated diene and hydroperoxide.

Estrada *et al.* (2001) reported that spirulina contains phycobiliproteins (phycocyanin and allophycocyanin). Studies were conducted to purify and characterize phycocyanin of *Spirulina platensis* and to demonstrate that one of the main components responsible for antioxidant activity in SP is a biliprotein phycocyanin. They studied the antioxidant activity of different fractions obtained during the phycocyanin purification process, through the scavenger activity of hydroxyl radical. They also observed that an increase in phycocyanin content was related to an increase in the antioxidant activity in different fractions, and therefore, phycocyanin is the component mainly responsible for the same activity.

2.1.6 Detoxification Properties

Venkataraman *et al.* (1994) evaluated ameliorative effect of *Spirulina platensis* on hexachlorocyclohexane (HCH) induced dietary toxicity in retinol-deficient male albino rats. Growth rate was considerably reduced in rats fed on vitamin A-free diets with and without HCH, while the body weight gain increased at the end of the seven weeks in rats fed on alga supplemented diets, with or without HCH. Alterations were discernible in serum and liver enzymes (glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and alkaline phosphate) of rats fed on diets containing HCH, but without the alga. Such changes were not observed in rats fed on HCH diets supplemented with alga. Results were suggestive of the ameliorating effects of alga on the dietary toxicity of HCH in retinol-deficient albino rats. Shastri *et al.* (1999) investigated the effect of *Spirulina fusiformis* @ 800 mg/kg (non toxic dose) on Lead toxicity in Swiss Albino mice. Lead toxicity affected the testes of mice. A significant enhancement in the survival time was observed in mice treated with *Spirulina fusiformis* compared with the Lead-treated control. Lead induced toxicity was also reduced in terms of testes weight, animal weight and tubular diameter. The modulatory effects of *Spirulina fusiformis* might be attributed to the presence of the antioxidants, carotene and SOD (superoxide dismutase).

Patni *et al.* (2001) opined that *Spirulina fusiformis* given @ 800 mg/kg body weight can be used as a detoxifying and modulating agent against toxicity induced by mercuric chloride in Swiss Albino mice where a mortality rate of 80% in forty eight hours at 6 mg/kg mercuric chloride was noticed. The mortality was only 60% at 30 days after treatment when 6 mg/kg mercuric chloride was applied with *Spirulina fusiformis* powder. Erythrocyte count and haemoglobin concentration were higher in the presence of *Spirulina fusiformis* compared to mercuric chloride-treated mice without *Spirulina fusiformis*

2.1.7 Haematopoietic Potentiating Properties

Kapoor and Mehta (1998) assessed the effect of spirulina on iron status based on hemoglobin, packed cell volume, serum iron, total iron binding capacity and ferritin levels of rats during pregnancy and lactation. Rats were fed five different kinds of diets (casein, spirulina, wheat gluten, spirulina + wheat gluten and spirulina without additional vitamins and minerals), each providing 22 percent protein. Diets containing spirulina alone or in combination with wheat gluten resulted in significantly higher iron storage and hemoglobin contents than casein and wheat gluten diets during the first half of pregnancy and lactation. Wheat gluten diet resulted in the smallest increase in haemoglobin levels and iron stores compared to other diets. The values of serum iron and iron binding capacity remained unchanged with different diets. Spirulina appears to be effective in improving the iron status of rats during pregnancy and lactation. Mani *et al.* (2000) studied the effect of supplementation of spray dried spirulina powder on blood haemoglobin levels in young anaemic girls and concluded that supplementation of spirulina had beneficial effect on the haemoglobin levels and could be effectively used to combat iron deficiency anaemia.

2.1.8 Anti-inflammatory and Anti-arthritic Properties

Vijayakumar and Venkataraman (2002) reported that *Spirulina platensis*, rich in beta-carotene and several micronutrients, could be therapeutically used in

the management of chronic refractory diseases in Indian medicine. In this study, the antiinflammatory and anti-arthritic properties of the blue-green algae *S. plantesis* were evaluated in rats. Hind paw oedema was induced using carrageenan, whereas arthritis was produced using an adjuvant. *Spirulina platensis*, at an oral dose of 1500 mg/kg, exhibited significant anti-inflammatory activity in the treated rats . In addition, SP administration significantly reduced the levels of lysosomal enzymes in both the acute and chronic phases of the inflammatory condition. These results supported the usefulness of SP as a food supplement in chronic refractory diseases like arthritis.

Remirez *et al.* (2002) studied the anti-inflammatory effects of microalgae Spirulina in zymosan-induced arthritis in mice. Four days after the intra-articular injection of zymosan (15 mg/ml), spirulina (100 and 400 mg/kg) was administered orally to animals for eight days. The mice were than killed and beta-glucuronidase was measured in the synovial fluid. Spirulina significantly reduced the levels of beta-glucuronidase that had been increased by zymosan. Histopathological and ultrastructural studies showed inhibition of the inflammatory reaction evidenced by intact cartilage, well-preserved chondrocytes, and normal rough endoplasmic reticulum and mitochondria

2.2 SPIRULINA SUPPLEMENTATION IN BIRDS

2.2.1 Growth Parameters

Ross and Dominy (1990) conducted three separate experiments to evaluate the nutritional qualities of *Spirulina platensis* in poultry. In experiment one, day old white leghorn male chicks (120 numbers) were fed isonitrogenous diets containing 0, 5, 10, 15 and 20% dried spirulina. At three weeks of age growth of chicks fed 10 and 20% spirulina was depressed. In experiment two, day old Hubbard male broiler chicks were fed with experimental diets containing 0, 1.5, 3, 6 or 12.5 % of spirulina. Although the growth of the chicks fed spirulina diets was not different from chicks receiving the control diets, birds receiving the

12% spirulina diet grew slower than the chicks fed all of other spirulina diets. In experiment three, one week old Japanese quails were used to study the effects of 0, 1.5, 3.0, 6.0 and 12.0 percentage of spirulina on growth, egg production, egg quality, fertility, hatchability and growth of F1 generation. There was no significant differences due to spirulina content in any of the parameters except for yolk colour, which increased with each succeeding levels of spirulina. However, fertility was higher for spirulina treatment. Venkataraman *et al.* (1994b) studied the replacement value of *Spirulina platensis* for fish meal and a vitamin-mineral premix for broiler chicks. The study was carried out for a period of 12 weeks by replacing either fish meal (FM) or groundnut cake (GC) in a commercial diet with algae at isonitrogenous concentrations of 140 g/kg and 170 g/kg, respectively. Efficiency of food utilisation, protein efficiency ratio and dressing percentage indicated that substitution of FM or GC by alga did not affect the performance of broilers. None of the diets affected the weights, composition and histology or microarchitecture of various organs of the chicks.

Baikovskaya *et al.* (2000) conducted a feeding trial in broiler chicken comprising five groups of 50 birds each. First group was fed on complete basal feed mixture without spirulina plus trace elements and cholicalciferol. Feed for second, third and fourth groups were supplemented with spirulina biomass at 0.05, 0.10 and 0.50% respectively plus trace elements and cholicalciferol. The last group was given basal diet supplemented with 0.50% spirulina biomass plus cholicalciferol only. Average daily weight gain was 34.1, 35.1, 36.1, 36.6 and 35.2 g, respectively for groups 1 to 5.

2.2.2 Immuno Modulating Properties

Qureshi *et al.* (1995) studied the effects of *Spirulina platensis* extract exposure on chicken macrophages. Spirulina-treated macrophages exhibited phenotypic changes in terms of increased spreading and vacuolization with minimal cytotoxicity. Percentage of phagocytic macrophages for unopsonised SRBC and average number of internalized SRBC were significantly higher in

spirulina-treated macrophages compared with sham-treated controls. However, phagocytosis of opsonized SRBC was not affected by spirulina treatment. Macrophage cultures exposed to SP produced a factor in their culture supernatant with tumoricidal potential which was similar in reactivity to the one produced by macrophages after exposure to lipopolysaccharide. The ability of splenic natural killer cells to kill tumour cell targets was not affected by SP treatment. The findings suggested that Spirulina exposure enhanced selected effector functions of cells of the chicken immune system after *in vitro* exposure.

Qureshi *et al.* (1996) studied the effect of *Spirulina platensis* supplementation on humoral and cell-mediated immune functions in chickens. Macrophages isolated from broilers of all spirulina groups had higher phagocytic potential than the control groups. Spirulina supplementation also increased NK-cell activity by two fold over the controls. These studies showed that spirulina supplementation can increase several immunological functions implying that a dietary inclusion of spirulina will enhance disease resistance potential in chickens.

When the effects of dietary *Spirulina platensis* on chicken macrophage phagocytic function and nitrite production was examined (Al-Batshan *et al.*, 2001) in all the spirulina dietary group, macrophages exhibited an enhanced phagocytic activity in terms of overall phagocytic percentage and the average number of SRBC per phagocytic macrophage. This increase was linear with each incremental increase of dietary spirulina. Data clearly showed that *S. plantesis* feeding regulates macrophage phagocytic as well as metabolic pathways leading to increased nitric oxide synthase activity.

2.2.3 Muscle and Yolk Pigmentation

Anderson *et al.* (1991) studied the effect of xanthophylls of spirulina on egg yolk pigmentation. Optimum pigmentation was obtained with 1% spirulina in a diet otherwise free of xanthophylls. Ross *et al.* (1994) compared freeze-dried

and extruded *Spirulina platensis* as yolk pigmenting agents. In an eight weeks long study, Japanese quails were given 0, 0.5, 1.0, 2.0 and 4.0% of freeze-dried spirulina or the dry equivalent of fresh spirulina extruded with maize. Another experiment of similar kind was conducted, but for an extended period of 16 weeks. Yolk colour increased with increasing dietary spirulina in both the experiments and there was a consistent increase with freeze-dried over the extruded spirulina. Venkataraman *et al.* (1994) found that meat had intense colour in the case of broiler birds fed on the spirulina containing diets.

Toyomizu *et al.* (2001) undertook a study to determine the effects of dietary spirulina on growth performance and pigmentation in the muscle of growing broiler chicken and found that zeaxanthin in spirulina might affected the development of yellow colour in meat. No significant differences among treatments were observed in body weight, nor weight or yield (as a percentage of body weight) for any of the selected traits, including liver, abdominal fat, kidney and pectoralis profundus. Spectrocolorimetric analyses revealed that the redness of pectoralis superficialis, profundus and sartorius muscles reached a maximum in chicks fed on spirulina diet, while the yellowness of all fillets, including the semitendinosus muscle, increased in a sub-linear fashion with increase of spirulina in the diet. The overall correlation between the yellowness and zeaxanthin content in the pectoralis muscle was significant. This study provided the first conclusive evidence that dietary spirulina influenced both the yellowness and redness of broiler flesh and that the increments in yellowness with dietary spirulina content might possibly be reflected in the common yellow pigment related to the accumulation of zeaxanthin within the flesh.

2.2.4 Anti-oxidant Parameters

Yuvraj *et al.* (2003) studied the role of supplementation of spirulina and natural carotenoids on augmenting the tissue antioxidative status in broiler chicken. Birds were divided into four groups. The first group was fed with normal broiler diet; feed for the second group was supplemented with spirulina at

1% level; in the third group natural carotenoids (soft gel, 10 mg/kg) supplemented along with 1 % spirulina in the feed and the fourth group fed with normal diet +10% coconut containing diet. Enzymatic antioxidants such as superoxide dismutase (SOD) and catlase (CAT) and non enzymatic antioxidant like reduced glutathione content as well as lipid peroxidation (LPO) level in 25% tissue homogenates of liver, ventricles of heart and brain were estimated. There were significantly higher activities of antioxidant enzymes as well as higher GSH content and decreased level of LPO in the tested tissues of spirulina and natural carotenoids-supplemented broilers compared to unsupplemented birds. High fat supplementation in the broiler ration resulted in a reverse effect on the tissue antioxidative parameters. The results substantiated that the supplementation of spirulina with and without natural carotenoids in poultry rations could augment the antioxidant activity in vital tissues.

Materials and Methods

3. MATERIALS AND METHODS

The experiment was conducted in egg type Austrowhite male chicken in the Department of Physiology, College of Veterinary and Animal Sciences, Mannuthy with an objective of evaluating the effect of supplementing blue green algae (*Spirulina plantesis*) on haematological, biochemical and fertility parameters for a period of twenty four weeks (from eight to thirty two weeks of age).

3.1 EXPERIMENTAL BIRDS

Thirty two, eight week old egg type Austro white male chicken were procured from University Poultry Farm, Mannuthy and were reared in battery cages under standard managemental conditions upto 32 weeks of age. The birds were selected randomly, weighed, wing banded and divided into two groups; G-I and G-II comprising of sixteen birds in each. Birds of group G-I were fed with standard layer ration while G-II group were fed on layer ration with spirulina supplemented at the rate of 2.5% of feed. Both rations were made isocaloric and isonitrogenous. Birds were provided with feed and clean drinking water *ad libitum*. The body weights of individual birds were recorded at monthly intervals till the end of the experiment (12, 16, 20, 24, 28 and 32 weeks).

3.2 EXPERIMENTAL RATION

The standard layer ration was formulated as per Bureau of Indian Standards specifications (BIS, 1992) and proximate principle was estimated as per Association of Official Analytical Chemists (AOAC, 1990). Though Spirulina was supplemented at the rate of 2.5% in the ration for G-II group, rations for both G-I and G-II were made isocaloric and isonitrogenic. The chemical composition and proximate analysis of the rations are presented in table

1a and 1b. The proximate analysis of feed and *Spirulina platensis* powder is presented in table 2 and figuratively represented in fig.2.

3.3 BLOOD COLLECTION

Blood samples were collected from wing vein with anticoagulant ethylene diamine tetra acetic acid (EDTA: 2 mg/ml blood) at monthly intervals from twelfth week to 32 weeks of age. Part of the blood was used for the estimation of haematological parameters and the remaining blood was centrifuged at 3000 rpm for 30 min to separate plasma. Plasma samples were aliquoted in labelled tubes and stored at -20^0 C for biochemical analysis.

3.4 HAEMATOLOGICAL PARAMETERS

Total erythrocyte count (TEC) and total leucocyte count (TLC) were estimated by the method suggested by Natt and Herrick (1952). Volume of packed red blood cells (VPRC) and erythrocyte sedimentation rate (ESR) were estimated on the day of blood collection as per standard procedures (Feldman *et al.*, 2000). The concentration of haemoglobin (Hb) was estimated by acid haematin method as described by Feldman *et al.* (2000). Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were calculated using standard formulae (Swenson and Reece, 1996).

3.5 BIOCHEMICAL PARAMETERS

3.5.1 Plasma Proteins

Total plasma proteins and plasma albumin were estimated by Biuret method as suggested by Henry *et al.* (1957) and Doumas method as described by Doumas *et al.* (1971) respectively using Ecoline ® kits (M/s. E. Merck India Limited, Mumbai).

Table 1a. Composition of egg type male chicken ration

Sl. No.	Ingredients	Standard Cockerel ration	
		Control	Experimental
1.	Yellow Maize	36	36
2	Rice polish	22	22
3	Wheat Bran	22	25
4	Ground nut cake	4	6.5
5	Soya bean meal	4	-
6	Spirulina powder	-	2.5
7	Gingley oil cake	4	-
8	Unsalted dried fish	6	6
9	Mineral mixture*	1.75	1.75
10	Salt	0.25	0.25
	Total	100.00	100.00
Added every 100 Kg			
11	Vitamin Mixture(AB2D3K)**	20g	20g
12	Coccidiostat***	50g	50g

* Keyes Mineral mixture (M/s Kerala Solvent Extraction Limited, Irinjalakkuda, Kerala, India):
Calcium 32%, Phosphorous 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,VitB2-50mg,Vit D3-12,000 IU and VitK-10 mg.

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

Table 1b. Proximate principle of egg type male chicken ration (on dry matter basis)

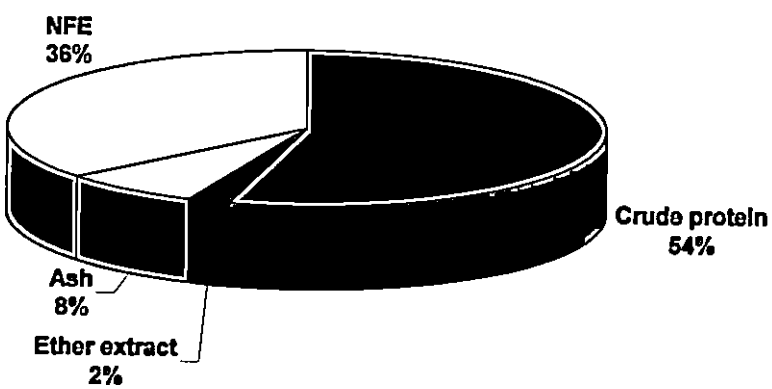
Sl No	Nutrients	Standard layer cockerel ration	
		Control	Experimental
Analysed values			
1	Moisture	9.68	10.7
2	Crude protein	16.4	16.8
3	Ether extract	4.87	5.92
4	Crude.fibre	6.28	6.71
5	Nitrogen free extract	47.32	42.06
6	Total Ash	11.5	13.8
7	Acid insoluble ash	3.92	4.01
Calculated values			
8	Metabolisable energy	2498.00	2584.00

Table 2. Proximate analysis of *Spirulina platensis* powder.

Sl No	Nutrients (Analysed values)	Percentage
1	Dry Matter	97*
2	Crude protein	54.26*
3	Ether extract	2.23*
4	Crude fibre	0*
5	Ash	7.53*
6	Nitrogen Free Extract	35.98*

* On dry matter basis.

Figure: 1.Proximate analysis of *Spirulina platensis* powder



The plasma globulin content was determined by subtracting plasma albumin level from total plasma protein content, assuming the fibrinogen level to be constant. A: G ratio was calculated subsequently from the values obtained.

3.5.2 Plasma Lipid Profile

Concentration of plasma total lipids was estimated by phosphovanilline method as described by Zoeliner (1962) using Labkit® kit (M/s. Labkit Spain).

Concentration of plasma triglycerides was estimated by the peroxidase coupled method suggested by Schettler and Nussel (1975) using Ecoline® kit (M/s. E. Merck India Limited, Mumbai).

The total cholesterol level was estimated by cholesterol phenol amino antipyrine (CHOD PAP) method, as suggested by Stockbridge *et al.* (1989) using Ecoline® kit (M/s. E. Merck India Limited, Mumbai).

3.5.3 Plasma Total Bilirubin Concentration

Total bilirubin concentration was estimated by the sulphanilic acid coupling method suggested by Schillong and Wende (1960) using Ecoline® kit (M/s. E. Merck India Limited, Mumbai).

3.5.4 Blood Urea Nitrogen Concentration

Blood Urea Nitrogen was estimated using the Di Acetyl Monoxime (DAM) method as suggested by Mayne (1994) using Dr. Reddy's Laboratories'® kit (M/s. Dr. Reddy's Laboratories, Hyderabad, India).

3.6 ANTIOXIDANT ACTIVITY

3.6.1 Plasma Catalase Activity

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

Reagents used

1. Phosphate Buffer 50mM, P^H 7

A) Dissolved 1.7g of KH_2PO_4 in double distilled water (DDW); made up to 250 ml

B) Dissolved 4.45g of $Na_2PO_4.2H_2O$ in 500 ml DDW or 3.549g Na_2PO_4 in 500 ml DDW

Mixed 250 ml of A and 387.5 ml of B

Stored at 2°C

2. Hydrogen peroxide fresh; 30mM

Diluted 0.34 ml (340 µl) of H_2O_2 with 100 ml phosphate buffer and stored at 2°C.

Procedure

Plasma was separated from heparinised blood by centrifugation and sediment RBC was washed three times with normal saline. To 200µl of thick sediment added 4 or 5 parts of DDW. The concentration of Hb of this solution was found to be below 5 g%. This became the stock solution. Ten microlitre of this haemolysate was taken and 5 ml of phosphate buffer was added.

Assay using spectrophotometer

Wavelength: 240 nm (Ultraviolet range)

	Phosphate buffer	Haemolysate	H_2O_2
Standard	1 ml	2 ml	-
Test	-	2 ml	1 ml

Recorded initial O.D and O.D at every 10s for 2 minutes. From the initial and final O.D, catalase activity was calculated using formulae given below.

$$\text{Activity in K/gm of Hb (Katal units)} = \frac{2.303}{T} \times \log \frac{[\text{first reading}]}{[\text{Second reading}]} \times \frac{a}{b} \times 1000$$

T=Time interval (10s)

a= Constant (value=1250)

b= Haemoglobin concentration ×10

3.6.2 Plasma Lipid Peroxidation

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

Reagents used

1. 20% Tri Chloro Acetic acid

2. 0.67% Thio Barbituric acid

Took 0.67g of TBA and added 100 ml of distilled water and heated slightly, without boiling.

Procedure

Taken 0.5ml of plasma. Added 2.5 ml of 20% of TCA and 1ml of 0.67% TBA. Mixed well and kept for 30 minutes in boiling water bath. Cooled under running tap water. Added 4ml of N-butanol and mixed well (rotor). Centrifuged at 1000 rpm for 5 minutes to separate organic layer. Absorbance of supernatant at 532 nm (Visible range) against blank (n-butanol) was taken using spectrophotometer and peroxidation level was found out using standard calibration curve constructed by using different known standard solutions.

3.7 SEMEN COLLECTION

The cocks were trained 3-4 times for ejaculation before actual collection of semen for measurement of semen quality. Three samples of semen were collected on 24th, 28th and 32nd week of experiment. Semen was collected as per the procedure of Burrows and Quinn (1937).

3.8 SEMEN EVALUATION

3.8.1 Physical Evaluation

3.8.1.1 Macroscopic Evaluation

The semen samples collected were examined just after collection for estimation of semen appearance score. It was scored from 1 to 5 by visual examination following standards described by Mc Daniel and Craig (1959).

Scoring Chart

Creamy	5
Thick milky	4
Milky	3
Thin milky	2
Watery	1

3.8.2 Microscopic Evaluation

3.8.2.1 Mass Activity and Motility

Motility and mass activity of the samples collected were estimated immediately as per the methods described by Roberts (1986).

3.8.2.1.1 Mass activity

Procedure

A drop of fresh semen was placed on a clean glass slide and examined under the low power of the microscope. Characteristic waves were observed. Depending on the speed and intensity of the waves the mass activity was graded as

0	No movements
+	Only individual movements
++	Slow waves and individual movements
+++	Fairly rapid waves
++++	Very rapid waves and dark thick waves

3.8.2.1.2 Motility

Procedure

Transferred one drop of diluted semen (1:10 dilution) on a clean glass slide and put a cover slip. Examined under high power of the microscope and assessed the percentage of progressively motile sperms.

Motility is usually expressed as:

Score	Grades	Percentage motility
0		No motility
1	Poor	Below 20% motility
2	Fair	20% to 40% progressive motility
3	Good	60% to 70% progressive motility
4	Very good	70% to 90% progressive motility
5	Excellent	Above 90% progressive motility

3.8.3 Estimation of Live and Dead Spermatozoa

3.8.3.1 Differential Staining

The staining of spermatozoa for differential count of live and dead spermatozoa was done following the method described by Lake and Stewart (1978) using Eosin and Nigrosine stain.

Procedure

One drop of 2% eosin and four drops of 10% nigrosine is mixed in a clean glass slide. One drop of semen sample was added to it and mixed gently and

Reagents used

Methylene Blue Solution

Dissolved 50 mg methylene blue in 100 ml of 2.9% sodium citrate buffer.

Procedure

Diluted 0.2 ml fresh, semen with 0.8 ml egg yolk citrate (1:4 dilutions) in a test tube at 37°C. Added 0.1ml methylene blue solution and mixed the contents. Covered the extended semen with half inch layer of liquid paraffin and placed the tube in a water bath at 46.5°C.Observed the change in colour of semen from greenish to creamy colour.

3.8.5 Resistance to cold and heat shock

The capacity of the spermatozoa to resist heat and cold shock was estimated as per the method described by Roberts (1986).

3.8.5.1 Cold Shock Resistance Test

Procedure

Evaluated the motility of freshly collected semen sample. Taken 0.5 ml of the collected semen sample and placed it in a beaker containing crushed ice for 10 minutes. Determined the percentage of live sperm and motility after cold shock.

3.8.5.2 Heat Shock Resistance Test

Procedure

Diluted the given sample of semen ten times with citrate buffer. Took 0.1 ml of the diluted semen in 7 test tubes and placed the tubes in a temperature controlled water bath at 46.5^{0}C. Examined the motility of the semen sample at

uniformly. From this mixture a moderately thick smear was made on clean glass slides, air dried and examined under oil immersion objective of the microscope. Counted about 300 spermatozoa (50 spermatozoa in each of six different microscopic fields). Unstained spermatozoa were categorized into live and stained or partially stained spermatozoa were counted as dead ones and percentage of live and dead sperms were calculated

3.8.3.2 Hypo Osmolarity Swelling Test

The test was done to assess the percentage of live and dead sperms in the collected samples as per the method suggested by Roberts (1986).

Reagents used

Hypo Osmolar Solution (HOS)

Dissolved 0.735 g sodium citrate and 1.351 g fructose in 100 ml distilled water. Aliquoted and stored at -200°C.This forms the hypoosmolar solution.

Procedure

Aliquot of HOS medium was thawed and warmed to 37°C. Added 1ml of HOS media to 0.1 ml of semen sample. Mixed well and incubated at 37°C for 30 min. Placed 10 µl HOS semen mixture in a clean glass slide and examined under the low power of the microscope. Spermatozoa with swollen tail ends were counted as live. Percentage of live spermatozoa in the collected sample was assessed by counting number of live spermatozoa from a total of 100 spermatozoa.

3.8.4 Estimation of Metabolic Activity of Spermatozoa

3.8.4.1 Methylene Blue Reduction Time (MBRT)

The Methylene Blue reduction time (MBRT) in minutes was estimated by the method described by Beck and Salisbury (1943).

Reagents used

Methylene Blue Solution

Dissolved 50 mg methylene blue in 100 ml of 2.9% sodium citrate buffer.

Procedure

Diluted 0.2 ml fresh, semen with 0.8 ml egg yolk citrate (1:4 dilutions) in a test tube at 37°C. Added 0.1ml methylene blue solution and mixed the contents. Covered the extended semen with half inch layer of liquid paraffin and placed the tube in a water bath at 46.5°C.Observed the change in colour of semen from greenish to creamy colour.

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3.8.5.2 Heat Shock Resistance Test

Procedure

Diluted the given sample of semen ten times with citrate buffer. Took 0.1 ml of the diluted semen in 7 test tubes and placed the tubes in a temperature controlled water bath at 46.5^{0}C. Examined the motility of the semen sample at

every 10 minutes interval and noted the time taken for cessation of the motility of sperms.

3.9 EXAMINATION OF TISSUES

3.9.1 Organ Weights

The birds were sacrificed at the end of the experiment and weights of liver, testes, spleen and pancreas were taken and expressed as percentage of body weight.

3.9.2 Tissue Antioxidant Activity

3.9.2.1 Tissue Peroxidation Level

Peroxidation levels of homogenized samples of liver, testes, pancreas and spleen were estimated as per the method described by Okhawa *et al.* (1979).

Reagents Used

TRIS-Hcl Buffer; 0.2 M, PH 7

Dissolved 24.2 grams of Tris in 800 ml distilled water. Adjusted the p^H with concentrated HCl and diluted to a final volume of one litre.

Procedure

Tissue sample (1 g) was taken in 3 ml of Tris-Hcl buffer. Homogenated the tissue homogenate mixture using a tissue homogenator. The homogenate mixture was filtered through a muslin cloth and the filtrate was used as the sample. Peroxidation levels of the tissues were estimated similar to the one adopted for plasma samples.

Results

4. RESULTS

4.1. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON BODY WEIGHT IN EGG TYPE MALE CHICKEN

Body weight of chicken of control G I group and spirulina-fed G II group are depicted in table 3 and figure 2. Body weight of experimental Austrowhite chicken (G II group) was significantly higher ($P < 0.05$) than birds in the control group (G I group) during the entire experimental period. Month wise comparison of body weight also revealed a significantly higher ($P < 0.05$) body weight for birds of G II group. In birds of control group (G I) varied significantly with each other and showed an increasing trend with the peak value reaching in the eighth month (2329.68 ± 11.46 g). Body weight of birds of G II group also differed significantly with each other and showed an increasing trend in body weight with the peak value reaching in the eighth month (2653.12 ± 11.6 g).

4.2. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON RELATIVE ORGAN WEIGHT IN EGG TYPE MALE CHICKEN

Relative organ weight of different organs of birds of control G I group and experimental G II group is given in the table 4 and figure 3. No significant difference was noted between the relative liver weight of birds in the two different groups G I and G II. The average values were 1.35 ± 0.01 g for G II group (spirulina-fed) and 1.36 ± 0.02 g for G I. group (control). Relative weight of spleen of G II group (spirulina-fed) was significantly higher ($P< 0.05$) with an average value of 0.15 ± 0.01 g (spirulina-fed) and 0.12 ± 0.01 g in birds of G I group (control). Relative weight of pancreas was significantly high for birds of G II group (0.36 ± 0.01 g) than birds of G I group (0.32 ± 0.01 g). Relative weight of testes also showed a significant difference between the two groups. It was

Table 3. Effect of dietary supplementation of *Spirulina platensis* on body weight in Austra-white male chicken from third to eighth month of age, mean ± S.E. (n = 16)

	Body weight (g)	
Age	Groups	
	G I(Control)	G II(Spirulina fed)
3rd month	$1012.5^{fx} \pm 18.68$	$1150.62^{fy} \pm 21.43$
4th month	$1243.75^{ex} \pm 19.83$	$1603.12^{ey} \pm 19.07$
5th month	$1528.12^{dx} \pm 18.66$	$1862.5^{dy} \pm 10.70$
6th month	$1814.06^{cx} \pm 20.02$	$2181.25^{ey} \pm 11.06$
7th month	$2078.12^{bx} \pm 12.04$	$2396.87^{by} \pm 13.28$
8th month	$2329.68^{ax} \pm 11.46$	$2653.12^{ay} \pm 11.60$
Overall	$1667.71^{x} \pm 13.26$	$1974.58^{y} \pm 12.74$

Mean ± SE (between ages) bearing different superscripts (a,b,c,d,e,f) in columns differ significantly ($P<0.05$)

Mean ± SE (between groups) bearing different superscripts (x,y) in rows differ significantly ($P<0.05$)

Table 4. Effect of dietary supplementation of *Spirulina platensis* on relative organ weight in Austra-white male eighth month of age, mean ± S.E. (n = 16)

	Relative Organ weights (g)	
Organ	Groups	
	G I(Control)	G II(Experimental)
Liver	$1.36^{x} \pm 0.02$	$1.35^{x} \pm 0.01$
Spleen	$0.12^{x} \pm 0.01$	$0.15^{y} \pm 0.01$
Pancreas	$0.32^{x} \pm 0.01$	$0.36^{y} \pm 0.01$
Testes	$0.85^{x} \pm 0.01$	$1.01^{y} \pm 0.02$

Mean ± SE (between groups) bearing different superscripts(x, y) in rows differ significantly ($P < 0.05$)

Figure: 2. Effect of dietary supplementation of *Spirulina platensis* on body weight of male Austra-white chicken from three to eight months of age

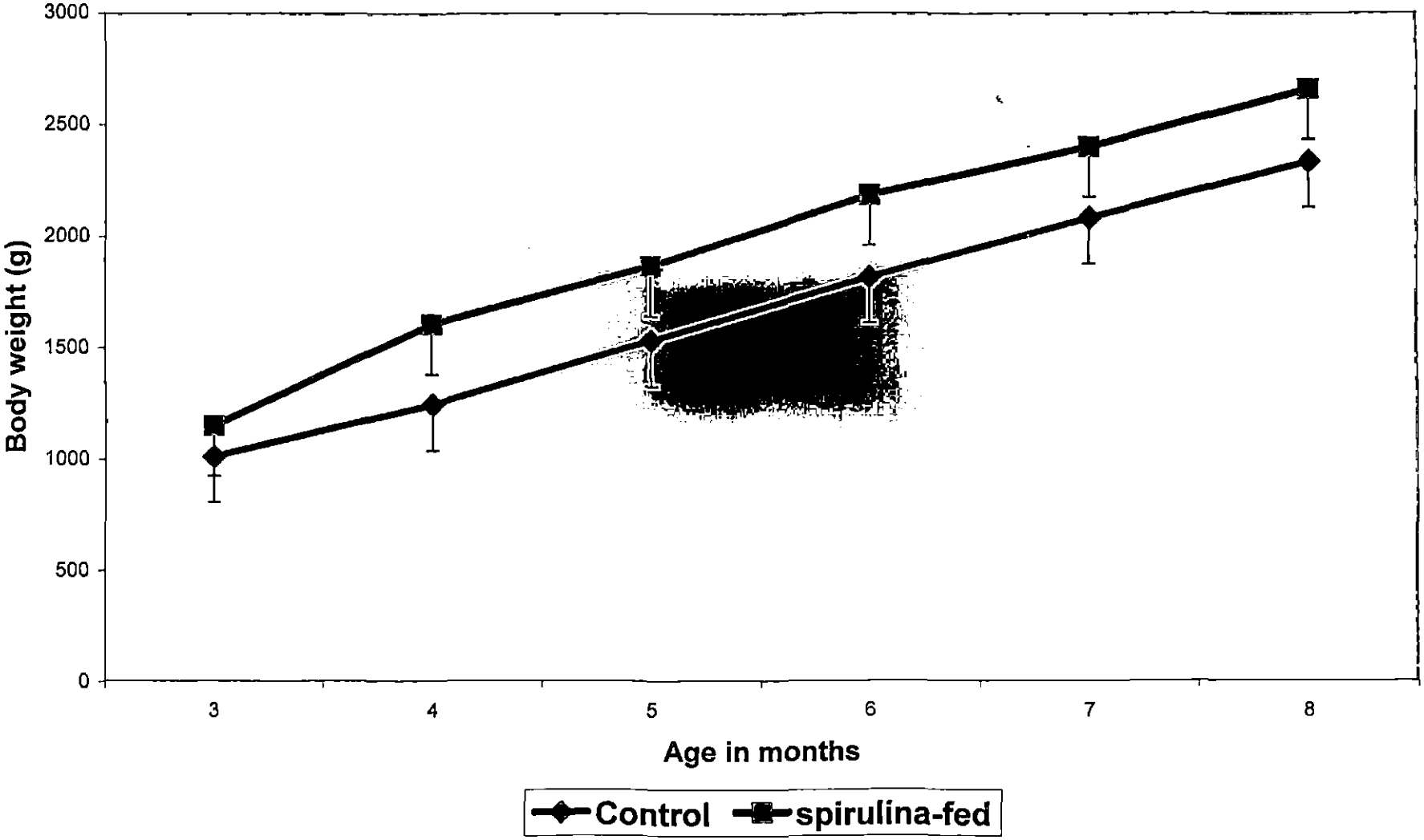
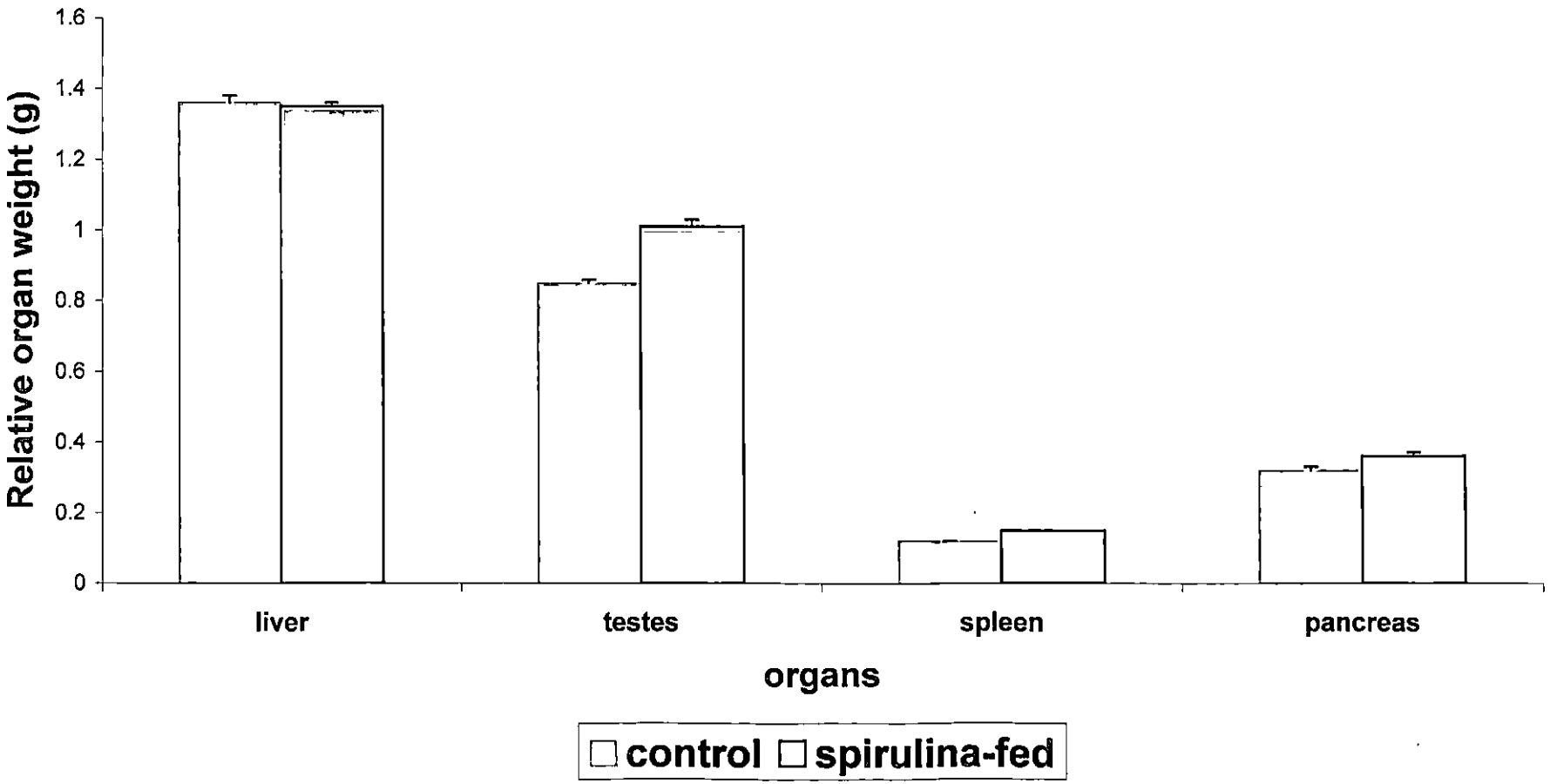


Figure:3. Effect of dietary supplementation of *Spirulina platensis* on relative organ weight in male Austra- white chicken at eighth month of age



significantly higher for birds of spirulina-fed group (G II) with an average value of 1.01 ± 0.02 g in G II and 0.85 ± 0.01 g in G I group (control).

4.3. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON HAEMATOLOGICAL PARAMETERS IN EGG TYPE MALE CHICKEN

The values of certain haematological parameters such as total erythrocyte count (TEC), haemoglobin (Hb) concentration, total leukocyte count (TLC), volume of packed red blood cells (VPRC), erythrocyte sedimentation rate (ESR), erythrocytic indices viz. mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of male Australowhite layer type chicken at monthly intervals and at the end of the experiment with and without dietary supplementation of blue green alga (*Spirulina platensis*) are depicted in tables 5a and 5b.

4.3.1. Total Erythrocyte Count (TEC)

Total erythrocyte count values were significantly higher ($P < 0.05$) for birds of G II group (spirulina-fed) during the entire experimental period. Month wise comparison of erythrocytic count also showed a significantly higher ($P < 0.05$) TEC for G II group in all the six months of study. The values showed an increasing trend in birds of control group (G I) all through the experimental period with the peak value of 3.53 ± 0.03 millions/µl reaching in the eighth month. Significant difference ($P < 0.05$) was seen between the TEC values of third, fourth, fifth, sixth and eighth month in birds of control group G I. No significant difference ($P > 0.05$) was observed between TEC values of sixth and seventh month in birds of G I group, but TEC value of seventh month differed significantly ($P < 0.05$) with that of all other months except with that of eighth month. TEC values of birds of G II group (spirulina-fed) also showed an increasing trend with the peak value of 4.02 ± 0.09 millions/µ attained in the

Table 5a. Effect of dietary supplementation of *Spirulina platensis* on haematological parameters in Austra-white male chicken from third to eighth month of age, mean ± S.E. (n = 16)

	TEC (millions/µl)		Hb (g %)		VPRC (%)		TLC (thousand/µl)		ESR (mm/ h)	
Groups / Age	G I	G II	G I	G II	G I	G II	G I	G II	G I	G II
3rd month	$2.33^{ex} \pm 0.05$	$3.06^{dy} \pm 0.04$	$10.81^{bx} \pm 0.29$	$13.18^{cy} \pm 0.24$	$25.81^{dx} \pm 0.36$	$30.12^{cy} \pm 0.27$	$22.00^{cx} \pm 0.51$	$23.12^{ax} \pm 0.56$	$2.75^{ax} \pm 0.17$	$1.75^{ay} \pm 0.17$
4th month	$2.80^{dx} \pm 0.03$	$3.76^{cy} \pm 0.10$	$11.93^{ax} \pm 0.34$	$14.12^{by} \pm 0.27$	$28.87^{cx} \pm 0.40$	$31.93^{by} \pm 0.38$	$23.37^{bcx} \pm 0.42$	$22.62^{ax} \pm 0.40$	$2.12^{bx} \pm 0.08$	$1.25^{by} \pm 0.11$
5th month	$3.28^{cx} \pm 0.04$	$3.97^{by} \pm 0.10$	$12.37^{ax} \pm 0.23$	$15.12^{ay} \pm 0.26$	$28.31^{cx} \pm 0.28$	$35.56^{ay} \pm 0.56$	$24.75^{abx} \pm 0.42$	$23.75^{ax} \pm 0.63$	$1.93^{bx} \pm 0.06$	$1.06^{bx} \pm 0.06$
6th month	$3.35^{bcx} \pm 0.04$	$4.125^{aby} \pm 0.12$	$12.56^{ax} \pm 0.22$	$14.68^{aby} \pm 0.25$	$31.06^{bx} \pm 0.417$	$35.37^{ay} \pm 0.51$	$24.81^{abx} \pm 0.46$	$23.43^{ax} \pm 0.54$	$1.56^{cx} \pm 0.12$	$1.00^{bx} \pm 0.00$
7th month	$3.48^{abx} \pm 0.03$	$4.02^{ay} \pm 0.09$	$12.75^{ax} \pm 0.19$	$14.56^{aby} \pm 0.24$	$31.06^{bx} \pm 0.49$	$36.00^{ay} \pm 0.50$	$25.12^{ax} \pm 0.23$	$22.93^{ay} \pm 0.80$	$2.00^{bx} \pm 0.00$	$1.12^{bx} \pm 0.08$
8th month	$3.53^{ax} \pm 0.03$	$4.05^{aby} \pm 0.11$	$12.37^{ax} \pm 0.22$	$14.87^{aby} \pm 0.25$	$32.68^{ax} \pm 0.58$	$35.31^{ay} \pm 0.42$	$24.50^{abx} \pm 0.35$	$23.12^{ax} \pm 0.85$	$1.43^{cx} \pm 0.12$	$1.06^{by} \pm 0.06$
Overall	$3.13^{x} \pm 0.03$	$3.87^{y} \pm 0.06$	$12.13^{x} \pm 0.12$	$14.42^{y} \pm 0.12$	$29.63^{x} \pm 0.29$	$34.05^{y} \pm 0.28$	$23.17^{x} \pm 0.19$	$24.10^{y} \pm 0.26$	$1.96^{x} \pm 0.13$	$1.21^{y} \pm 0.16$

G I -Control group; G II –Spirulina-fed group

Mean ± SE (between ages) bearing different superscripts (a, b, c, d, e, f) in columns differ significantly ($P < 0.05$)

Mean ± SE (between groups) bearing different superscripts (x, y) in rows differ significantly ($P < 0.05$)

seventh month. Significant difference ($P < 0.05$) was observed between the TEC values in third, fourth, fifth and seventh months of age in birds of G II group. No significant difference ($P > 0.05$) could be observed between TEC values of sixth, seventh and eighth month in birds of G II group.

4.3.2. Haemoglobin Concentration

The values of haemoglobin concentration were significantly higher ($P < 0.05$) for birds of G II group compared to G I group throughout the experimental period. Month wise comparison also showed a significant variation ($P < 0.05$) between the haemoglobin concentration values of birds of G I and G II groups in all the months. Haemoglobin values in the G I group (control) showed a fluctuating trend with the peak value attaining during the seventh month (12.75 ± 0.19 g %). There was no significant difference in the haemoglobin values of different months in birds of G I group except for the third month which was significantly lower ($P < 0.05$) when compared to other months. Haemoglobin values of the different months of G II group (spirulina-fed) also showed a fluctuating trend with the peak value attaining in the fifth month (15.12 ± 0.26 g %). Haemoglobin values of the first and second months G II group differed significantly ($P < 0.05$) with other months and between themselves also.

4.3.3. Volume of Packed Red Cells (VPRC)

Volume of packed red cells was significantly higher ($P < 0.05$) for birds of G II group compared to G I group throughout the experimental period. Month wise comparison of VPRC values of the two groups also revealed a significantly higher ($P < 0.05$) VPRC values for G II group in all the months of study. VPRC increased progressively in G I group with highest value shown in the eighth month (32.68 ± 0.58 %) which varied significantly ($P < 0.05$) than the values of all other months. VPRC values showed a fluctuating trend in G II with a maximum value recorded in the seventh month (36 ± 0.50 %).The values of

VPRC values of G II group (spirulina-fed) at fifth, sixth, seventh and eighth month did not vary significantly ($P > 0.05$), but they varied significantly ($P < 0.05$) at third and fourth month.

4.3.4. Erythrocyte Indices

4.3.4.1. Mean Corpuscular Volume (MCV)

The values of mean corpuscular volume were significantly higher ($P < 0.05$) for birds of G I group during the entire period of experiment. Month wise comparison between G I and G II showed a significantly higher ($P < 0.05$) MCV values for the third and fourth months. No significant difference ($P > 0.05$) in MCV values were seen between the two groups except the third and fourth month. In birds of G I group the MCV values showed a fluctuating trend with the lowest value recorded in the fifth month (86.39 ± 5.47 fl). In G I, MCV values of third and fourth month significantly differed between each other and also with the MCV values of rest of the months. No significant difference was noted between MCV values of sixth, seventh and eighth month in birds of G I group. The value of fifth month did not differ significantly with seventh month, but differed significantly in all the other months. In birds of G II group also, the MCV values showed a fluctuating trend with the lowest value seen in the fourth month (83.62 ± 5.73 fl). The values of third month showed a significant difference with the MCV values of all the other months, but no significant difference was seen between MCV values of fourth, fifth, sixth seventh and eighth month in birds of G II group.

4.3.4.2. Mean Corpuscular Haemoglobin (MCH)

The values of MCH did not showed any significant difference ($P > 0.05$) between the two groups for the entire experimental period. Month wise comparison of MCH values of birds in G I and G II groups did not show any

Table 5b. Effect of dietary supplementation of *Spirulina platensis* on erythrocytic indices in Austra-white male chicken from third to eighth month of age, mean ± S.E. (n = 16)

	MCV (fl)		MCH (pg)		MCHC (g%)	
Groups / Age	G I	G II	G I	G II	G I	G II
3rd month	$111.64^{ax} \pm 3.40$	$98.51^{ay} \pm 1.71$	$47.22^{ax} \pm 1.70$	$43.11^{abx} \pm 1.01$	$41.98^{abx} \pm 1.21$	$43.80^{dx} \pm 0.80$
4th month	$103.53^{bx} \pm 6.74$	$83.62^{by} \pm 5.73$	$42.68^{abx} \pm 2.79$	$37.97^{bcx} \pm 2.70$	$41.49^{abx} \pm 2.85$	$44.23^{ay} \pm 2.78$
5th month	$86.39^{dx} \pm 5.47$	$90.25^{bx} \pm 5.77$	$31.42^{dx} \pm 3.74$	$47.28^{ay} \pm 3.77$	$43.73^{ax} \pm 2.69$	$42.68^{abx} \pm 2.69$
6th month	$93.03^{cx} \pm 5.82$	$86.79^{bx} \pm 5.62$	$37.52^{bcx} \pm 2.89$	$36.22^{cx} \pm 4.77$	$40.36^{bcx} \pm 2.48$	$41.68^{abx} \pm 2.65$
7th month	$89.22^{cdx} \pm 5.60$	$85.96^{bx} \pm 5.24$	$36.59^{cdx} \pm 2.24$	$34.92^{cx} \pm 2.17$	$41.22^{abx} \pm 2.60$	$40.56^{bx} \pm 2.51$
8th month	$92.68^{cx} \pm 1.93$	$88.14^{bx} \pm 2.88$	$35.07^{cdx} \pm 2.63$	$37.24^{cx} \pm 1.50$	$38.02^{cx} \pm 2.89$	$42.16^{aby} \pm 2.67$
Overall	$91.04^{x} \pm 1.53$	$87.44^{y} \pm 1.28$	$35.37^{x} \pm 0.92$	$36.64^{y} \pm 0.87$	$39.09^{x} \pm 0.38$	$41.84^{y} \pm 0.45$

G I -Control group;G II –Spirulina-fed group
Mean ± SE (between ages) bearing different superscripts (a,b,c,d,e,f) in columns differ significantly ($P < 0.05$)
Mean ± SE (between groups) bearing different superscripts (x,y) in rows differ significantly ($P < 0.05$)
fl- femto litre ; pg- Pico gram

significant difference in any months except for the fifth month which was significantly higher ($P < 0.05$) for birds of G II group. In birds of G I group MCH values showed a decreasing trend with the highest value seen in the first month (47.22 ± 1.70 pg). The birds of G II group showed a fluctuating trend in MCH values with the peak value reported in the fifth month (47.28 ± 3.77 pg).

4.3.4.3. Mean Corpuscular Haemoglobin Concentration (MCHC)

The values of MCHC were significantly higher ($P < 0.05$) for birds of G II group for the entire experimental period. Month wise comparison showed a significantly higher ($P < 0.05$) MCHC values for birds of G II group in fourth and eighth month of the experiment. No significant difference was noted between MCHC values in any other months of study. In birds of G I group, MCHC values showed a fluctuating trend with the lowest value seen in the eighth month (38.02 ± 2.89 g%) which differed significantly with the MCHC values of all other month except with that of sixth month. Birds of G II group also showed a fluctuating trend in MCHC values with the lowest value reported in the seventh month (40.56 ± 2.51 g%).

4.3.5. Erythrocyte Sedimentation Rate (ESR)

The value of ESR in birds of G II group was significantly lower ($P < 0.05$) than that of G I group during the entire experimental period. Month wise comparison of ESR showed a significantly lower ($P < 0.05$) values for G II in third, fourth and eighth month when compared to G I. Fifth, sixth and seventh month did not show any significant difference in the ESR values between birds of G I and G II group. In birds of G I group the value of ESR was maximum in the third month (2.75 ± 0.17 mm/h), which decreased gradually in the coming months and reached a lowest value of 1.43 ± 0.12 mm/h in the eighth month. .ESR in G II showed a fluctuating trend with the lowest ESR value recorded in the sixth month (1.00 ± 0 mm/h). The value of ESR in birds of G II group in third

month showed a significant difference with that of other months, but no significant difference in was noted between values of ESR of fourth, fifth, sixth, seventh and eighth months.

4.3.6. Total Leukocyte Count (TLC)

Total leukocyte count values were significantly higher ($P < 0.05$) for birds of G II group (spirulina-fed) compared to control birds of G I group during the entire experimental period. But a month wise comparison of TLC revealed no significant difference ($P > 0.05$) between G I and G II except for the seventh month which showed a significant difference between the two groups. It showed an increasing trend in G I then dipped in last month with the peak value reaching in the seventh month (25.12 ± 0.23 thousands/mm^3). Total leukocyte count of third month in G I differed significantly ($P < 0.05$) with that of all other months except that of fourth month. In birds of G II group, the TLC of different months did not show any significant differences ($P > 0.05$).

4.4. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON BLOOD BIOCHEMICAL PARAMETERS IN EGG TYPE MALE CHICKEN

4.4.1. Plasma Protein Profile

4.4.1.1. Total Plasma Protein

Effect of supplementation of blue green algae (*Spirulina platensis*) on total plasma protein concentration of the two groups from third to eight month of age is given in table 6. Plasma total protein level was significantly higher ($P<0.05$) for birds of G II group during the entire experimental period. Month wise comparison of total proteins also showed a significantly higher plasma total protein level in birds of G II group in different months of study. Total protein

Table 6. Effect of dietary supplementation of *Spirulina platensis* on plasma protein profile in Austra-white male chicken from third to eighth month of age, mean ± S.E. (n = 16)

	Total proteins (g /dl)		Albumin (g /dl)		Globulin (g /dl)		A: G ratio	
Groups / Age	G I	G II	G I	G II	G I	G II	G I	G II
3rd month	4.17^{dx} ± 0.06	5.10^{cy} ± 0.07	1.86^{dx} ± 0.02	2.11^{cy} ± 0.02	2.31^{cx} ± 0.06	2.99^{dx} ± 0.06	0.81^{ax} ± 0.02	0.71^{ay} ± 0.01
4th month	4.37^{cx} ± 0.06	5.22^{dey} ± 0.05	1.88^{cdx} ± 0.01	2.03^{cy} ± 0.04	2.49^{bx} ± 0.05	3.19^{bcy} ± 0.02	0.76^{abx} ± 0.01	0.63^{by} ± 0.01
5th month	4.59^{cx} ± 0.05	5.36^{dy} ± 0.03	1.94^{bdx} ± 0.02	2.22^{by} ± 0.03	3.64^{bx} ± 0.06	3.13^{cdy} ± 0.04	0.74^{bx} ± 0.02	0.71^{ay} ± 0.02
6th month	4.63^{bx} ± 0.05	5.49^{cy} ± 0.07	1.97^{bcx} ± 0.02	2.22^{by} ± 0.05	2.65^{bx} ± 0.05	3.26^{bcy} ± 0.05	0.75^{bx} ± 0.02	0.68^{aby} ± 0.02
7th month	5.02^{ax} ± 0.04	5.70^{by} ± 0.11	2.00^{bx} ± 0.02	2.36^{ax} ± 0.06	3.02^{ax} ± 0.05	3.34^{by} ± 0.01	0.67^{cx} ± 0.02	0.71^{ay} ± 0.03
8th month	5.09^{ax} ± 0.03	5.99^{ay} ± 0.08	2.13^{ax} ± 0.02	2.42^{ax} ± 0.01	2.95^{ax} ± 0.04	3.56^{ay} ± 0.08	0.72^{bcx} ± 0.01	0.68^{aby} ± 0.02
Overall	4.65^{x} ± 0.84	5.48^{y} ± 0.93	1.96^{x} ± 0.14	2.23^{y} ± 0.19	2.68^{x} ± 0.12	3.25^{y} ± 0.25	0.74^{x} ± 0.09	0.69^{y} ± 0.12

G I -Control group; G II –Spirulina-fed group

Mean ± SE (between ages) bearing different superscripts (a,b,c,d,e,f) in columns differ significantly ($P < 0.05$)

Mean ± SE (between groups) bearing different superscripts (x,y) in rows differ significantly ($P < 0.05$)

level of G I showed an increasing during the different months of study with the maximum value attained in the eighth month (5.09 ± 0.03 g/dl). No significant difference (P > 0.05) was seen in total protein levels in seventh and eighth month and also between fourth and fifth month in birds of G I group, but significant difference was seen in all the other months. In birds of G II group also total protein levels showed an increasing trend with maximum value seen in the eighth month (5.99 ± 0.08 g/dl). No significant difference was seen in total protein levels of third, fourth month and fourth and fifth month. Total protein levels of all the other months differed significantly with each other in G II.

4.4.1.2. Plasma Albumin

Effect of supplementation of blue green algae (*Spirulina platensis*) on albumin concentration of the two groups from third to eighth month of age is given on table 6. Albumin level of birds of G II group was significantly higher (P < 0.05) than birds of G I group during the entire experimental period. Month wise comparison of albumin value also showed a significantly higher (P < 0.05) value for G II group except for the seventh and eighth month which did not vary significantly between the two groups. Albumin level in birds of G I group showed an increasing trend through out the experimental period with the peak value attained in the eighth month (2.13 ± 0.02 g/dl). No significant difference in the albumin value could be appreciated in fifth, sixth and seventh month in birds of G I group the value of eighth month differed significantly with that of all other months. In G II group, albumin level increased uniformly from third month onwards with a peak value of 2.42 ± 0.01 g/dl shown in the eighth month although no significant difference in the albumin values was seen between seventh and eighth month, but differed significantly with other months vide table 6.

4.4.1.3. Plasma Globulin

Effect of supplementation of blue green algae (*Spirulina platensis*) on globulin concentration of the two groups from third to eighth months of age is given on table 6. Globulin level was significantly higher ($P < 0.05$) for birds of G II group during the entire experimental period. Although no significant difference ($P < 0.05$) was shown between globulin level of birds of G I and G II in the third month, but in other months there was significant difference ($P < 0.05$). Globulin levels in birds of G I group showed an increasing trend up to seventh month with the peak value reaching in the seventh month (3.02 ± 0.05 g/dl). In birds of G I group, albumin levels of seventh and eighth month did not show any significant difference between each other, but they differed significantly with other months. Globulin levels of third, fourth, fifth and sixth months also showed insignificant difference between each other, but differed significantly with that of first month. In birds of G II group, levels showed an increasing trend with the peak levels obtained in the eighth month (3.56 ± 0.08 g/dl). Globulin levels of birds of G II group differed significantly ($P < 0.05$) in eighth month with that of all other months.

4.4.1.4. Albumin: Globulin Ratio (A: G ratio)

Effect of supplementation of blue green algae (*Spirulina platensis*) on A: G ratio of the two groups from third to eighth month of age is given on table 6. The value of A: G ratio of birds of G I group was significantly higher than birds of G II group for the entire experimental period. Monthly comparison of A: G ratio also showed a significant difference between birds of G I and G II groups in different months. The A: G ratio was maximum in the third month of study (0.81 ± 0.02) in G I group and it differed significantly with A: G ratios of all other months except at fourth month. The A: G ratio of birds of G II group showed a fluctuating trend with a maximum value showed in the seventh month of study (0.71 ± 0.03).

4.4.2. Plasma Lipid Profile

4.4.2.1. Plasma Total Lipids

Effect of supplementation of blue green algae (*Spirulina platensis*) on concentration of plasma total lipids of the two groups from third to eighth month of age is given on table 7. Total lipids were significantly lower for birds of G II group during the entire experimental period. Month wise comparison also revealed a significantly lower total lipid level in birds of G II group compared to G I group in the different months of study. In birds of G I group total lipid level increased gradually from third month and reached a peak value of 512.97 ± 1.23 mg/dl in the eighth month. Significant differences ($P < 0.05$) were found in the total lipid level of different months except for the fifth and sixth month, which did not show any significant difference between each other. In birds of G II group there was a gradual reduction in total lipid levels which reached a minimum value in the eighth month (425.29 ± 0.88 mg/dl). In birds of G II group total lipid level of third and fourth month did not differ significantly with each other as was the case of total lipid level in fifth, sixth, seventh and eighth month.

4.4.2.2. Plasma Triglycerides

Effect of supplementation of blue green algae (*Spirulina platensis*) on triglyceride concentration of the two groups from third to eighth month of age is given on table 7. Birds of G II group showed a significantly higher ($P < 0.05$) triglyceride level than control birds during the entire experimental period. Monthly comparison of the triglyceride level between G I and G II showed a significantly higher ($P < 0.05$) level of triglycerides in G II in all the months of experiment except for the eighth month. In birds of G I group triglyceride level increased progressively and reached a maximum value of 94.28 ± 0.46 mg/dl in the eighth month. No significant difference was noted in the triglyceride levels of third and fourth month in birds of G I group, but triglyceride levels of third

Table 7. Effect of dietary supplementation of *Spirulina platensis* on plasma lipid profile in Austra-white male chicken from third to eighth month of age, mean ± S.E. (n = 16)

	Plasma total lipids (mg/dl)		Triglycerides (mg/dl)		Cholesterol (mg/dl)	
Groups / Age	G I	G II	G I	G II	G I	G II
3rd month	$484.03^{ex} \pm 2.64$	$447.02^{ay} \pm 1.87$	$82.35^{cx} \pm 0.81$	$102.42^{ay} \pm 0.74$	$184.03^{ex} \pm 1.57$	$136.2^{bcy} \pm 2.60$
4th month	$497.15^{dx} \pm 2.38$	$444.38^{ay} \pm 1.35$	$87.48^{bcx} \pm 0.35$	$102.23^{ay} \pm 0.46$	$196.37^{dx} \pm 2.23$	$133.18^{cy} \pm 2.55$
5th month	$502.09^{cx} \pm 2.69$	$436.20^{by} \pm 1.50$	$89.48^{abx} \pm 0.35$	$102.35^{ay} \pm 0.38$	$218.90^{cx} \pm 3.20$	$132.12^{cy} \pm 2.61$
6th month	$505.36^{cx} \pm 2.60$	$432.43^{bcy} \pm 1.14$	$90.83^{abx} \pm 0.39$	$102.67^{ay} \pm 1.00$	$221.98^{cx} \pm 2.23$	$134.63^{cy} \pm 2.75$
7th month	$512.00^{bx} \pm 2.00$	$428.86^{cdy} \pm 1.00$	$93.23^{abx} \pm 0.57$	$103.23^{ay} \pm 0.69$	$228.37^{bx} \pm 2.39$	$140.48^{by} \pm 3.15$
8th month	$512.97^{ax} \pm 1.23$	$425.29^{dy} \pm 0.88$	$94.28^{ax} \pm 0.46$	$99.73^{ax} \pm 1.39$	$234.86^{ax} \pm 2.09$	$146.19^{ay} \pm 4.05$
Overall	$503.43^{x} \pm 1.96$	$435.7^{y} \pm 1.28$	$89.61^{x} \pm 1.32$	$102.14^{y} \pm 1.65$	$214.08^{x} \pm 345$	$137.15^{y} \pm 3.98$

G I -Control group; G II –Spirulina-fed group

Mean ± SE (between ages) bearing different superscripts (a, b, c, d, e, f) in columns differ significantly ($P < 0.05$)

Mean ± SE (between groups) bearing different superscripts (x, y) in rows differ significantly ($P < 0.05$)

month differed significantly ($P < 0.05$) with all the other months. In birds of G II group, triglyceride levels remained more or less uniform through out the experimental period except for the last month which was slightly lower when compared to other months (99.73 ± 1.39 mg/dl). But no significant difference ($P > 0.05$) was found between triglyceride levels of different months in G II.

4.4.2.3. Plasma Cholesterol

Effect of supplementation of blue green algae (*Spirulina platensis*) on the plasma cholesterol concentration of the two groups from third to eighth months of age is given on table 7. The cholesterol level was significantly lower in birds of G II group during the entire experimental period. In birds of G I group, cholesterol level increased progressively during the different months of study reaching a maximum value of 234.86 ± 2.09 mg/dl in the eighth month. In birds of G II group, cholesterol level showed a decreasing trend up to sixth month and reached the lowest level of 134.63 ± 2.75 mg/dl. The plasma levels of cholesterol of birds in G II group at seventh and eighth month were significantly higher ($P < 0.05$). Maximum cholesterol level was observed in the eighth month with the level reaching 146.19 ± 4.05 mg/dl.

4.4.3. Blood Urea Nitrogen (BUN)

Effect of supplementation of blue green algae (*Spirulina platensis*) on BUN concentration of the two groups from third to eighth months of age is given in table 8. The level of BUN was significantly higher for birds in G II group during the entire experimental period. Monthly comparison of the level of BUN in G I and G II also showed significantly higher levels in G II in all the months of study. In birds of G I group, BUN values increased convincingly with a maximum level shown in eighth month of study (3.44 ± 0.03 mg/dl). Birds in G II also showed an increasing trend and the maximum level was noted in eighth month of study (4.40 ± 0.07 mg/dl). In G II, although no significant difference

Table 8. Effect of dietary supplementation of *Spirulina platensis* on concentration of blood urea nitrogen (BUN) and plasma bilirubin in Austra-white male chicken from third to eighth month of age, mean ± S.E. (n = 16)

	Blood urea nitrogen (mg/dl)		Total bilirubin (mg/dl)	
Groups / Age	G I	G II	G I	G II
3rd month	2.94 ± 0.04^{ex}	3.81 ± 0.07^{by}	0.44 ± 0.01^{ax}	0.57 ± 0.01^{ax}
4th month	3.10 ± 0.03^{dx}	3.73 ± 0.07^{bcy}	0.44 ± 0.01^{ax}	0.57 ± 0.01^{ax}
5th month	3.20 ± 0.03^{cdx}	3.67 ± 0.07^{cy}	0.44 ± 0.01^{ax}	0.57 ± 0.00^{ax}
6th month	3.25 ± 0.03^{bcx}	3.84 ± 0.07^{by}	0.45 ± 0.01^{ax}	0.58 ± 0.01^{ax}
7th month	3.34 ± 0.03^{abx}	4.32 ± 0.07^{ay}	0.44 ± 0.01^{ax}	0.57 ± 0.01^{ax}
8th month	3.44 ± 0.03^{ax}	4.40 ± 0.07^{ay}	0.44 ± 0.01^{ax}	0.58 ± 0.01^{bx}
Overall	$3.21^{x} \pm 0.15$	$3.96^{y} \pm 0.09$	$0.44^{x} \pm 0.02$	$0.58^{y} \pm 0.05$

G I -Control group; G II –Spirulina-fed group

Mean ± SE (between ages) bearing different superscripts (a, b, c, d, e, f) in columns differ significantly ($P < 0.05$)

Mean ± SE (between groups) bearing different superscripts (x, y) in rows differ significantly ($P < 0.05$)

was noted between BUN levels of seventh and eighth month of study, they both differed significantly with BUN levels of all the other months.

4.4.4. Bilirubin

Effect of supplementation of blue green algae (*Spirulina platensis*) on plasma concentration of bilirubin of the two groups from third to eighth months of age is given on table 8. The bilirubin value was significantly higher for birds in G II during the entire experimental period. Month wise comparison did not show any significant difference between birds in G I and G II groups in any months of study. In birds of both G I and G II groups levels of plasma bilirubin did not vary significantly within the group during different months of study.

4.5. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON ANTIOXIDANT STATUS IN EGG TYPE MALE CHICKEN

4.5.1. Blood Antioxidant Status

4.5.1.1. Blood Catalase Activity

The catalase activity of blood two groups of bids G I and G II from three to eight months are presented in table 9a and figure 4. Blood catalase activity was significantly higher ($P < 0.05$) in birds of G II for the entire experimental period. Month wise comparison also showed a significantly higher catalase activity in group G II except for the third month which did not show any significant difference between G I and G II. In birds of G I group blood catalase activity showed a fluctuating trend with the peak activity noted in sixth month (7.69 ± 0.08 k/g Hb). In G II catalase activity showed an increasing trend with the peak activity noted in eighth month (9.56 ± 0.03 k/g Hb). No significant difference

Table 9a. Effect of dietary supplementation of *Spirulina platensis* on blood antioxidant status in Austra-white male chicken from third to eighth month of age, mean ± S.E. (n = 16)

	Plasma lipid peroxidation (nmol/ml homogenate)		Blood catalase (k/g Hb)	
Groups / Age	G I	G II	G I	G II
3rd month	$3.30^{cx} \pm 0.06$	$2.86^{ay} \pm 0.08$	$7.23^{cx} \pm 0.11$	$7.75^{dx} \pm 0.15$
4th month	$3.58^{bx} \pm 0.06$	$2.75^{aby} \pm 0.06$	$7.37^{bcx} \pm 0.08$	$8.93^{cy} \pm 0.06$
5th month	$3.68^{abx} \pm 0.05$	$2.64^{bcy} \pm 0.05$	$7.25^{cx} \pm 0.11$	$9.15^{bcy} \pm 0.02$
6th month	$3.44^{cx} \pm 0.08$	$2.56^{cy} \pm 0.05$	$7.69^{ax} \pm 0.08$	$9.32^{aby} \pm 0.04$
7th month	$3.78^{ax} \pm 0.04$	$2.33^{dy} \pm 0.02$	$7.60^{abx} \pm 0.10$	$9.53^{ay} \pm 0.05$
8th month	$3.71^{abx} \pm 0.06$	$2.38^{dy} \pm 0.02$	$7.34^{cx} \pm 0.12$	$9.56^{ay} \pm 0.03$
Overall	$3.58^{x} \pm 0.14$	$2.58^{y} \pm 0.09$	$7.42^{x} \pm 0.13$	$9.05^{y} \pm 0.05$

G I -Control group; G II –spirulina-fed group
Mean ± SE (between ages) bearing different superscripts (a, b, c, d, e, f) in columns differ significantly ($P < 0.05$)
Mean ± SE (between groups) bearing different superscripts (x, y) in rows differ significantly ($P < 0.05$)

Figure :4. Effect of dietary supplementation of *Spirulina platensis* on blood catalase level in male Austra-white chicken from three to eight months of age

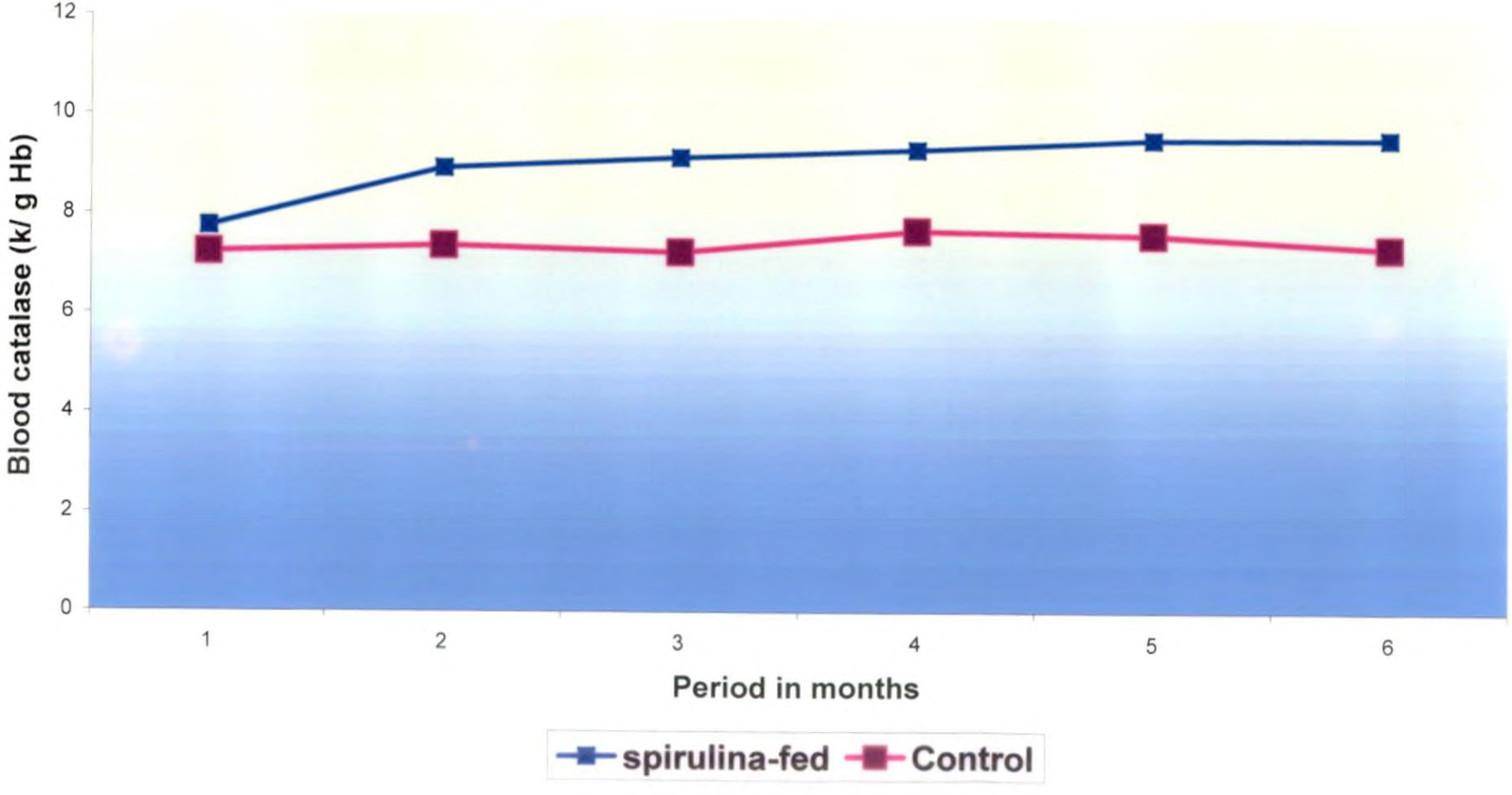
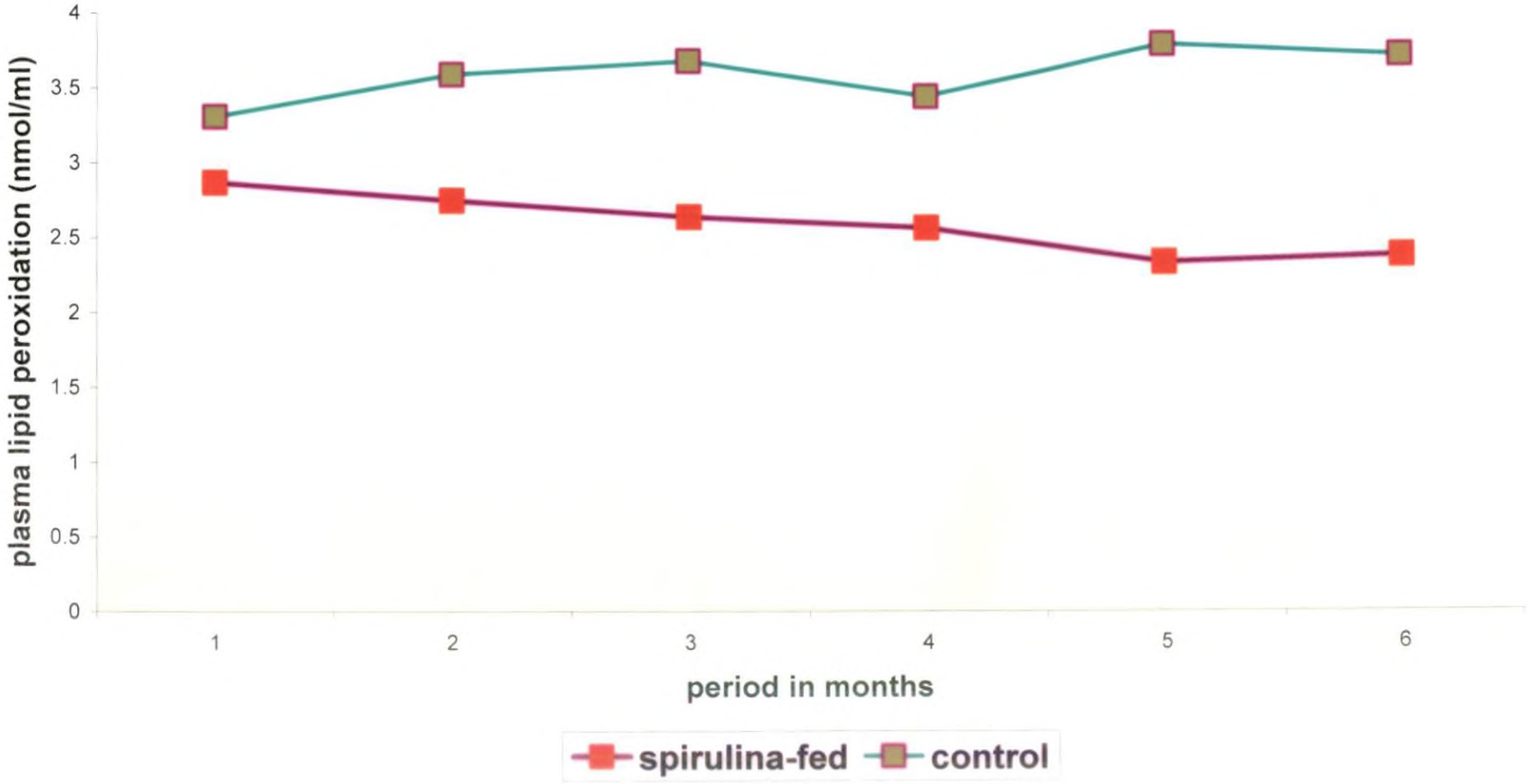


Figure:5. Effect of dietary supplementation of *Spirulina platensis* on plasma lipid peroxidation level of male Austra-white chicken from three to eight months of age



(P>0.05) was noted between catalase activities of sixth, seventh and eighth month in G II.

4.5.1.2. Plasma Lipid Peroxidation Level

Plasma lipid peroxidation level of two groups from three to eight months is presented in table 9a and figure 5. Plasma lipid peroxidation levels were significantly lower ($P < 0.05$) in G II (spirulina-fed) for the entire experimental period. Month wise comparison also showed a significantly lower peroxidation levels in birds of G II group in all the months of study. Plasma peroxidation levels in G I showed a fluctuating trend with the peak value obtained in seventh month (3.78 ± 0.04 nmol/ml). Plasma peroxidation levels in G II showed a decreasing trend with the level reaching lowest in seventh month. Plasma peroxidation level of seventh and eighth month in birds of G II differed significantly with that of all other months, but did not differ significantly between each other.

4.5.2. Tissue Peroxidation Level

The effect of spirulina supplementation on tissue peroxidation levels in egg type male chicken is depicted in table 9b and figure 6. Peroxidation levels of homogenized liver samples of birds from birds of G II group were significantly lower ($P < 0.05$) than birds of G I group with an mean value of 3.13 ± 0.06 nmol/ml for birds in G II and 4.98 ± 0.08 nmol/ml for birds in G I. No significant difference was noted between the peroxidation levels in homogenized samples of spleen of birds of G I group and birds of G II group. The average values were 1.25 ± 0.03 nmol/ml for birds in G II and 1.28 ± 0.04 nmol/ml for birds in G I. No significant difference was noted between the peroxidation levels in homogenized samples of pancreas of birds of G I group and birds of G II group. The average values were 1.32 ± 0.04 nmol/ml for birds in G II and 1.28 ± 0.04 nmol/ml for birds in G I. Peroxidation levels of homogenized testes samples of

Figure:6. Effect of dietary supplementation of *Spirulina platensis* on tissue peroxidation level in Austra-white male chicken at eight months of age

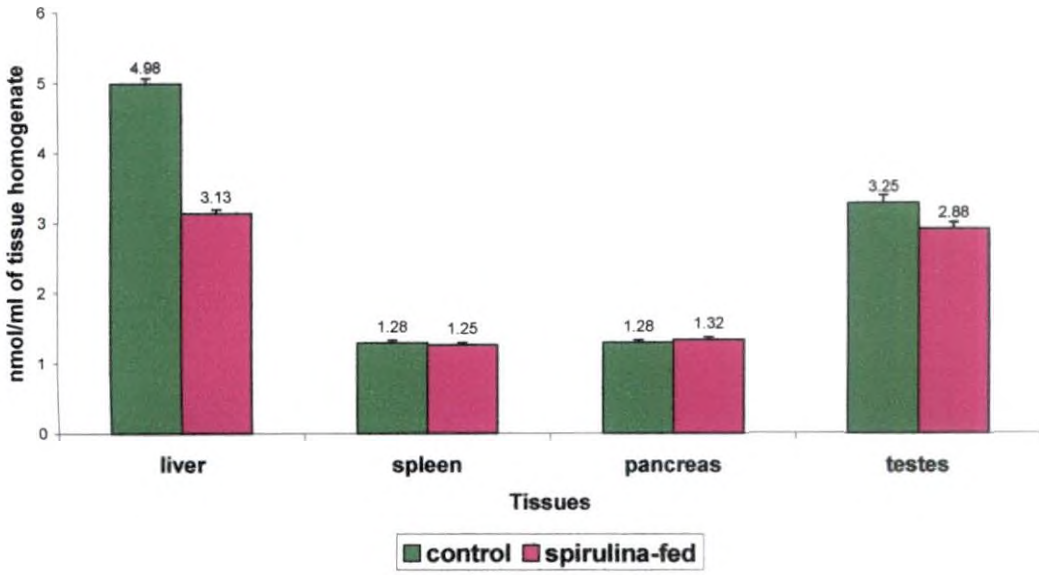


Table 9b. Effect of dietary supplementation of Spirulina platensis on tissue peroxidation level in Austra-white male chicken at eighth month of age, mean ± S.E. (n = 16)

Organ	Tissue peroxidation level (nmol/ml homogenate)	
	Groups	
	G I(Control)	G II(Experimental)
Liver	$4.98^{x} \pm 0.08$	$3.13^{y} \pm 0.06$
Spleen	$1.28^{x} \pm 0.04$	$1.25^{x} \pm 0.03$
Pancreas	$1.32^{x} \pm 0.04$	$1.28^{x} \pm 0.04$
Testes	$2.88^{x} \pm 0.09$	$3.25^{y} \pm 0.11$

Mean ± SE (between groups) bearing different superscripts(x, y) in rows differ significantly ($P < 0.05$)

birds from G II were significantly lower ($P < 0.05$) than G I with an average value of 2.88 ± 0.09 for birds of G II group and 3.25 ± 0.11 nmol/ml for birds of G I group.

4.6. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON FERTILITY PARAMETERS (EXAMINATION OF SEMEN) IN EGG TYPE MALE CHICKEN

4.6.1. Gross Evaluation Score

Semen samples were evaluated and scored based on the external appearance immediately after collection. The scores for the two groups are given in Table 10a. Average semen evaluation scores was found to be higher for birds in G II in all the three collections.

4.6.2. Motility

The diluted semen samples were scored from 1 to 5 depends upon the motility for the diluted sample. The scores for the two groups in different months are given in Table 10a. The scores were found to be higher for birds in G II group up to eight months of age.

4.6.3. Mass Activity

Mass activity of the semen samples in different months are presented in Table 10a. Mass activity of the semen samples did not show any difference between the two groups in various months of study

Table 10a. Effect of dietary supplementation of *Spirulina platensis* on fertility parameters (1) in Austra-white male chicken from sixth to eighth month of age, mean ± S.E. (n = 16)

	Gross evaluation score		Motility (score)		Mass activity (grades)	
Groups / Age	G I	G II	G I	G II	G I	G II
6th month	(Thin milky) 2.75	Thick milky (4.06)	(60 to 70 %) 3.06	(70 to 90 %) 4.37	rapid waves (+++)	rapid waves (+++)
7th month	(Thin milky) 2.87	(Milky) 3.87	(60 to 70 %) 3.00	(70 to 90 %) 4.5	rapid waves (+++)	rapid waves (+++)
8th month	(Milky) 3.00	(Thick milky) 4.00	(60 to 70 %) 3.00	(70 to 90 %) 4.56	rapid waves (+++)	rapid waves (+++)

G I -Control group; G II –Spirulina-fed group

4.6.4. Semen Volume

Semen from birds belonging to both G I and G II groups collected on 24th, 28th and 32nd week of age and semen volume of the birds is given on Table 10b. Semen volume was found to be significantly higher for birds of G II during the entire collection periods with an overall average value of 0.53 ± 0.03 for birds in G II and 0.28 ± 0.20 for birds in G I. Month wise comparison also revealed a significantly higher ($P < 0.05$) semen volume for birds in G II in all the three months. In birds of G I semen volume did not vary significantly ($P > 0.05$) in different months of study where as in G II group there was significant variation ($P < 0.05$) with that of third month.

4.6.5. Methylene Blue Reduction Time (MBRT)

Methylene Blue reduction time of various semen samples collected are given in table 10b. Methylene Blue reduction time of birds in G II was significantly lower ($P < 0.05$) than G I during the entire period of semen collection with an average value of 4.29 ± 0.15 in birds of G II group and 5.35 ± 0.32 for birds of G I group. Month wise comparison also showed a significantly lower ($P < 0.05$) time in G II group in all the months of study. MBRT did not vary significantly between different months in both G I and G II groups

4.6.6. Estimation of Live and Dead Sperms

4.6.6.1. Differential Staining

The percentage of live sperms obtained by the differential staining of live sperms of the various semen samples collected is given in Table 10b.G II showed a significantly higher percentage of live sperms in differential staining during all the three collections (sixth, seventh and eighth month). The overall average was found to be 93.93 ± 0.62 percentage for birds in G II group and 83.95 ± 0.58

Table 10b. Effect of dietary supplementation of *Spirulina platensis* on fertility parameters (2) in Austra-white male chicken from sixth to eighth month of age, mean ± S.E. (n = 16)

	Ejaculate volume(ml)		Methylene Blue Reduction Time(min)		Differential staining (% of live sperms)		Hypoosmolarity test (% of live sperms)	
Groups / Age	GI	GII	GI	GII	GI	GII	GI	GII
6th month	$0.291^{ax} \pm 0.01$	$0.494^{by} \pm 0.03$	$5.625^{ax} \pm 0.12$	$4.063^{ay} \pm 0.17$	$83.37^{ax} \pm 0.35$	$94.75^{ay} \pm 0.56$	$83.12^{ax} \pm 0.34$	$93.12^{ay} \pm 0.68$
7th month	$0.269^{ax} \pm 0.01$	$0.538^{aby} \pm 0.5$	$5.063^{ax} \pm 0.06$	$4.438^{ay} \pm 0.18$	$83.81^{ax} \pm 0.61$	$93.25^{by} \pm 0.62$	$83.58^{ax} \pm 0.72$	$91.81^{ay} \pm 0.72$
8th month	$0.284^{ax} \pm 0.01$	$0.581^{ay} \pm 0.02$	$5.375^{ax} \pm 0.31$	$4.375^{ay} \pm 0.17$	$84.58^{ax} \pm 0.64$	$93.81^{aby} \pm 0.45$	$83.81^{ax} \pm 0.43$	$91.93^{ay} \pm 0.95$
Overall	$0.28^{x} \pm 0.2$	$0.53^{y} \pm 0.03$	$5.35^{x} \pm 0.32$	$4.29^{y} \pm 0.15$	$83.95^{x} \pm 0.58$	$93.93^{y} \pm 0.62$	$83.54^{x} \pm 0.51$	$92.92^{y} \pm 0.83$

G I -Control group; G II –Spirulina-fed group

Mean ± SE (between ages) bearing different superscripts (a, b, c, d, e, f) in columns differ significantly ($P < 0.05$)

Mean ± SE (between groups) bearing different superscripts (x, y) in rows differ significantly ($P < 0.05$)

percentage for birds of G I group. Month wise comparison also revealed a significantly higher percentage of live sperms in birds of G II group in all the three months of collection. No significant difference was seen in birds of G I group between the different months of semen collection .In birds of G II group significant difference was found in the percentage of live sperms in semen samples collected in 24th and 28th weeks. But semen samples collected in the 32nd week did not show any significant difference between the samples of 24th or 28th week of age.

4.6.6.2. Hypoosmolarity Swelling Test

The percentage of live sperms obtained through Hypoosmolarity swelling test of various semen samples is given in Table 10b. G II showed a significantly higher ($P < 0.05$) percentage of live sperms compared to G I in all the three collections. Average percentage of live sperms in G II was 92.92 ± 0.83 for birds in G II and 83.54 ± 0.51 for birds in G I. Month wise comparison also revealed a significantly higher percentage of live sperms in birds of G II group.

4.6.7. Heat Shock

Average Heat shock resistance time of the semen samples of the two groups are presented in Table 10 c. No significant difference was seen between the heat shock resistance times of semen samples from the two groups in the entire three months of collection. Average time in minutes for the complete loss of motility was found to be 28.43 ± 0.74 minutes in G II group and 27.79 ± 0.54 minutes in G I group. Month wise comparison also showed no significant difference between the two groups in the resistance time

Table 10c. Effect of dietary supplementation of *Spirulina platensis* on fertility parameters (3) in Austra-white male chicken from sixth to eighth month of age, mean ± S.E. (n = 16)

	Resistance of sperms			
	Heat (time in minutes)		Cold (% of live sperms)	
Groups / Age	GI	GII	GI	GII
6th month	$28.75^{ax} \pm 0.55$	$26.87^{bx} \pm 0.78$	$62.06^{ax} \pm 0.53$	$61.06^{ax} \pm 1.2$
7th month	$30.00^{ax} \pm 0.81$	$29.06^{abx} \pm 0.81$	$61.06^{ax} \pm 0.44$	$60.50^{ax} \pm 1.1$
8th month	$30.63^{ax} \pm 0.62$	$29.37^{ax} \pm 1.1$	$60.75^{ax} \pm 0.62$	$61.06^{ax} \pm 1.18$
Overall	$29.79^{x} \pm 0.54$	$28.43^{y} \pm 0.74$	$61.29^{x} \pm 0.45$	$60.87^{y} \pm 0.91$

G I -Control group; G II –Spirulina-fed group
Mean ± SE (between ages) bearing different superscripts (a, b, c, d, e, f) in columns differ significantly ($P < 0.05$)
Mean ± SE (between groups) bearing different superscripts (x, y) in rows differ significantly ($P < 0.05$)

4.6.8. Cold Shock

Average motility of the semen samples of the two groups after cold shock are presented in Table 10c. No significant difference was seen in the percentage of live sperms after the induction of cold shock between the two groups. Average Month wise comparison also showed no significant difference in the percentage of live sperms between the two groups

Discussion

5. DISCUSSION

5.1. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON BODY WEIGHT IN EGG TYPE MALE CHICKEN

Body weight of birds of G II group (spirulina-fed) were significantly higher in all the months of study (table 3; figure 2). Spirulina is a highly nutritious food with very high protein content. Protein content can reach up to 65-77% on dry matter basis. Protein from spirulina contains 18 amino acids, major ones being lysine, arginine, threonine, methionine and phenylalanine. The absence of thick cell wall, typical of plant and algal cells may contribute to the high digestibility coefficient of spirulina protein (Mepham, 1997). Proximate analysis conducted by Yoshida and Hoshii (1990) to evaluate the nutritive value of spirulina has shown that freeze-dried samples of spirulina contained 55.87% crude protein, about 2% crude fat and 3.29 Kcal/g available energy. Further, Blum *et al.* (1976), Yoshida and Hoshi (1990), and Becker and Venkataramanan (1982) fed varying levels of spirulina to growing chickens with satisfactory body weight gain at 5 to 10 percentage levels while growth was depressed at levels above 20%. Similar results were obtained when spirulina was fed to laying hens (Nazerenko *et al.*, 1975)). Ross and Dominy (1990) fed diets of 50 to 150 g/kg dehydrated spirulina to growing chicken with relatively satisfactory results for growth. Venkataraman *et al.* (1994) also showed that substitution of groundnut protein with sun dried and powdered spirulina up to 170g/kg or of fish meal with spirulina up to 140 g/kg on an equal protein basis resulted in satisfactory performance.

Results obtained in the present study is underlining the strong nutritious properties of spirulina and proves beyond doubt that spirulina can be used as a dietary supplement for chicken

5.2. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON RELATIVE ORGAN WEIGHT IN EGG TYPE MALE CHICKEN

Relative organ weight is the weight of the organ per 100g of body weight. Relative organ weight can give an idea about the differential growth of the subject in a test situation as to the predilection of a treatment to various organs in the body. In the present context it tells whether the spirulina supplementation had any direct influence on the organ concerned.

In the present experiment relative weight of spleen, pancreas and testes were significantly higher for G II group (spirulina-fed). But, relative weight of liver did not show any significant difference between the two groups (table 4; figure 3).

Increase in spleen weight might be due to the increase in the total erythrocyte count in the experimental group receiving dietary spirulina since spleen is the principal site where aged erythrocytes are destroyed and removed from circulation. In addition, the increase could have been attributed to an elevated immune status by virtue of more phagocytic cells in the circulation produced by spleen. However, direct observations supporting this view have not been attempted in the present study. On the other hand, elevated relative pancreas weight might be an indirect consequence of an increased responsiveness to the insulinogenic effect of high arginine content of spirulina (second most abundant amino acid, that is next to leucine, Yoshida and Hoshii, 1980), mediated by growth hormone (Ganong, 1996).

Higher relative weight of testes in spirulina-fed group indicated that the alga can have a fertility augmenting effect. Detailed examination of other fertility-related parameters supported enough.

5.3. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON HAEMATOLOGICAL PARAMETERS IN EGG TYPE MALE CHICKEN

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.

5.3.1. Haematopoietic Effect

5.3.1.1 TEC, Hb Concentration, VPRC and Erythrocytic Indices

In the present study, total erythrocyte count (TEC) of chicken receiving spirulina supplementation (G II group) was significantly higher than that of control bird (G I group) vide table 5a. Similarly, blood haemoglobin content and volume of packed red cells (VPRC) of G II group (spirulina-fed) was significantly higher than G I group (controls) in all the months of study (table 5a). The increase in value of VPRC in birds of G II group can be directly attributed to the increased erythrocyte count in these birds.

Along with the above results, calculated erythrocytic indices from the corresponding values of erythrocyte count, haemoglobin content and VPRC revealed a profound haematopoietic effect conferred by the blue green alga to its recipients. Mean corpuscular volume (MCV), was significantly lower for birds of G II during the entire experimental period (table 5b). While haemoglobin content of an average erythrocyte in the body, the value of mean corpuscular haemoglobin (MCH), did not show any significant difference between the two groups, mean corpuscular haemoglobin concentration (MCHC), the amount of haemoglobin per 100 ml of erythrocyte volume was significantly higher for G II group (spirulina-fed) vide table 5b.

Thus, it is indicated that there was a more marked increase in total erythrocyte count with moderate increase in the haemoglobin content of spirulina-fed cockerels over the control birds. Statistically insignificant increase in MCH values in spirulina fed birds of group II over the controls is a result of this discrepancy. The discrepancy may indicate the fact that spirulina brings forth its erythropoietic effect in the cockerels heavily by stimulating mitotic activity of the haemopoietic stem cells or by increasing the average life span of red cells, far more than other mechanisms such as augmenting expression of globins and accumulation/ conservation of iron. However, this propensity changed as the age of the bird advanced, since the synergistic effects of innate growth factors, if any, would be different in adulthood.

The salient features of these findings were in agreement with Patni *et al.* (2001) who recorded a higher erythrocytic count and haemoglobin concentration in mice supplemented with spirulina. Similarly, studies conducted by Mani *et al.* (2000) concluded that spirulina had beneficial effect on haemoglobin levels and supplementation of spray-dried spirulina powder could be effectively used to combat iron deficiency anaemia. When the effect of spirulina in pregnant and lactating rats based on haemoglobin concentration, packed cell volume, serum iron, total iron binding capacity and ferritin levels was studied Kapoor and Mehta (1998) observed that spirulina appeared to be effective in improving the iron status of rats during such physiological conditions. Studies conducted by Liu and Zhang (2002) showed an up-regulation by spirulina of expressions of hematopoietic cells, supposedly by way of promoting endogenous cytokine secretion. Findings of all these earlier studies are indicative of a strong haematopoietic effect of spirulina.

In fact, erythropoiesis is influenced by many nutritional factors; protein in general, many vitamins like vitamin B_{12}, pyridoxine, riboflavin, nicotinic acid, pantothenic acid, thiamine, biotin, and ascorbic acid and minerals such as iron, cobalt, and zinc, all have positive influence on erythropoiesis (Swenson and

Reece 1996). Whereas spirulina is a rich source of protein, many of these vitamins and minerals (Blinkova *et al.* 2001), an increased erythrocyte count, blood haemoglobin content and VPRC in the spirulina-fed cockerels could be a testimony to its anti-anaemic effect in birds in general and the chicken in particular. All the same, the exact mechanism involved, especially predilections this alga can have with reference to age and endogenous growth factors are yet to be resolved.

5.3.2. Erythrocytic Sedimentation Rate (ESR)

ESR can be a measure of the presence and intensity of disease process in the body (Wintrobe, 1974). Extreme fluctuations in ESR can indicate deviation from normal health status. In this sense this parameter can be of some value in assessing the welfare of the subject in a test situation. Since the avian erythrocytes are biconvex in shape the cells show little tendency to sediment due to poor rouleux formation. This could be the reason why Arun and Lokhande (1994) stated that ESR would not be a useful test in case of birds. Nevertheless, Sturkie (1976) has studied the sedimentation of chicken erythrocytes and has found that the mean sedimentation rates mostly fall between 1.5 and 4 mm/h with a possible range from 0.5 to 9 mm/h. Most of the values reported in the present study are in the former range. Moreover, ESR was significantly lower for birds of G II group (spirulina-fed, table 5a) than for birds of control group.

ESR in mammals is largely influenced by many factors like plasma proteins, TEC, cell size etc. (Benjamin, 1985). Since ESR is inversely proportional to TEC and erythrocyte size the decreased rate of erythrocyte fall in birds of G II group can be due to the increased erythrocyte count and decreased erythrocyte size (MCV). Also, hypercholesteraemia and hyperglobulinaemia have been shown to increase ESR in mammals (Benjamin, 1985). In the present experiment, since spirulina supplementation rendered a hypocholesteraemic effect lower values of ESR in spirulina-fed birds are consistent with the findings

in mammals; never withstanding higher levels of globulin in this group seemingly did not have similar influence upon ESR. In the present experiment, hypocholesteraemia concurrent with lower values of ESR in spirulina-fed birds is consistent with the findings in mammals. However, higher levels of globulin in this group seemingly did not have similar influence upon ESR.

5.3.3. Total Leukocyte Count (TLC)

Although, in the present study month wise comparison of total leukocyte count (TLC) in the two groups did not show any significant difference, in the entire experimental period the values of TLC was significantly higher in birds of G II group than the G I group (table 5a). Studies have proved that spirulina can improve both cell mediated and humoral immunity (Hayashi *et al.,* 1994).

5.4. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON BLOOD BIOCHEMICAL PARAMETERS IN EGG TYPE MALE CHICKEN

5.4.1. Plasma Protein Profile

The levels of plasma total protein, albumin and globulin were significantly higher ($P < 0.05$) for birds of G II group (spirulina-fed) during the entire experimental period (table 6). Sturkie and Newman (1951) observed a total protein level of 4 g%, albumin level of 1.66 g% and globulin level of 2.33 g% in adult male chicken. Values obtained in the present study were slightly higher in both control group (G I) and spirulina fed group (G II)

Many factors like stage of hydration or dehydration, haemorrhage, level of protein nutrition, sex and stage of development influence the level of plasma protein in birds (Leveille and Sauberlich, 1961). Chemical and nutritional analysis of spirulina was done by Mepham (1997) and reported very high protein

content, especially with high biological value. More importantly, absence of thick cell wall, typical of plant and algal cells, may contribute to the high digestibility coefficient of spirulina protein. Thus, high plasma protein content observed in G II can be attributed to the high protein content of spirulina with superior digestibility and biological value.

The levels of albumin and globulin also showed a significantly higher value in birds of G II. Albumin is believed to act as a protein reserve and a protein source at times of subnormal intake. In their normal role, however, albumins also act as carriers of many nutrients, including mineral elements, vitamins and fatty acids. In the chicken, albumins have an additional function as carriers of thyroid hormones (Sturkie, 1976), the latter being important in rendering its permissive action for augmenting the growth process. Therefore, higher levels of plasma albumin, as conferred by spirulina in this study, can be beneficial to the growing birds.

The globulins consist of four main fractions viz. α_1, α_2, β, and γ globulins. Gamma globulins, also known as immunoglobulins are mainly involved in immunologic reactions. Spirulina is a potent immunomodulatory agent in a sense that it positively influence the production of immunoglobulins in the body (Hayashi *et al.*, 1994). An increase in the globulin fraction of plasma protein may underline the above mentioned property of spirulina. However, only a fractional estimation of plasma globulins can confirm this point.

5.4.2. Plasma Lipid Profile

Plasma concentrations of total lipids and cholesterol were significantly lower for birds of G II group (spirulina-fed) during the entire experimental period (table 7). These results were in agreement with earlier studies on hypolipidaemic property (Iwata, 1990; Parikh *et al.*, 2001; Samuels *et al.*, 2002) and

hypocholesteraemic property (Amudha and Premakumari 1996; Kato and Takemoto 1984) of spirulina.

The levels of triglyceride were significantly higher for G II group during the entire experimental period (table 7). During periods of mobilisation of adipose tissue fatty acids in the form of free fatty acids (FFA) one of the many fates these FFA can have is their esterification in liver to yield triglycerides (Cunningham, 1997). It is then possible that the elevated triglyceride levels observed in the experimental group of cockerels in the present study has been the result of such esterification, which in turn, imply lipolytic effect of the alga.

5.4.3. Blood Urea Nitrogen (BUN)

Urea is the major nitrogenous end product of protein metabolism in mammals where as in birds uric acid is the excretory vehicle for four-fifths of metabolized nitrogen. Blood urea nitrogen (BUN) level was significantly higher for spirulina-fed birds of G II group during the entire experimental period (table 8).

Higher levels of BUN can be due to a variety of reasons. In, in the present experiment increased BUN in G II group cockerels can be due to a better plane of nutrition bestowed on these birds by a higher score of digestibility and biological value of spirulina protein, as evidenced by significantly higher body weight gain for all the months of study (table 3; figure 2).

5.4.4. Bilirubin

Level of plasma bilirubin was within the normal range of 0.2 to 0.8 mg/dl as reported by Sturkie (1976) and was significantly higher for birds in G II group during the entire experimental period (table 8). Bilirubin is the excretory product formed from the catabolism of heme of Hb. Accordingly, higher plasma bilirubin

level parallels with increased erythrocyte number and blood haemoglobin content in the experimental group.

5.5. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON ANTIOXIDANT STATUS IN EGG TYPE MALE CHICKEN

5.5.1. Blood Antioxidant Status

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

Blood catalase activity was significantly higher in G II group (spirulina-fed) for the entire experimental period while the plasma lipid peroxidation levels were significantly lower in G II group (table 9a; figure 4). 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 the free radical scavenging ability of the 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 II group (spirulina-fed) in the present study indicates a better antioxidant status in these birds.

Free radicals generated from many routine biochemical reactions in the body are involved in normal physiological process in the living organisms. They act as messengers for signal transduction and also affect the gene expression

(Armario *et al.*, 1990). Body's inherent mechanisms try to reduce the injury induced by free radicals by enzymatic and non-enzymatic ways and higher levels of antioxidants have been correlated with decreased susceptibility to cell damage (Pillai and Pillai, 2002). Several proteins and other biomolecules in the living system can act as free radical scavengers in the sense that these molecules are capable of containing the harmful squeal of excessive free radicals. Beside these endogenous molecules several dietary supplements containing vitamins, polyphenols and flavons also are found to play a similar scavenging role (Sherman, 2000). When the concentration of these scavenging biomolecules remains optimum imminent physiological irregularities are minimised. On the other hand, when free radicals' output increases beyond manageable limits physiological/biochemical disorders ensue (Cotgreave *et al.*, 1988). Plasma peroxidation levels of spirulina fed group were significantly lower (table 4-8; figure 5). The results indicate the role of spirulina in free radical scavenging activity and hence its effectiveness as a potent antioxidant agent.

5.5.2. Tissue Antioxidant Activity

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of diseases. Actually, it is involved in the formation and propagation of lipid radicals, uptake of oxygen and rearrangement of unsaturated lipids yielding a variety of degraded products like alkanes, malondialdehyde (MDA), conjugated dienes and lipid hydroperoxides, all such products being harmful to the membrane lipids (Moran *et al.* 1979). Excessive lipid peroxidation can also cause deteriorating changes in cellular metabolism of tissues culminating in cellular degradation and ultimately cell death (Winrow *et al.*, 1993).

Many antioxidants of plant origin are used for effective protection of various tissues against oxidative stress (Nishigaki *et al.*, 1992; Khan *et al.*, 1996). Spirulina contains phenolic acids, tocopherol and beta carotene, which are known to exhibit antioxidant properties (Miranda *et al.*, 1998). Peroxidation levels of

homogenized samples of liver and testes of G II group (spirulina-fed) birds were significantly lower. Homogenised samples of spleen and pancreas did not show any significant difference in the peroxidation levels. Peroxidation levels as such were very low in the homogenised samples of spleen and pancreas of birds from both the groups (figure 6).

Yuvraj *et al.* (2003) studied the role of supplementation of spirulina and natural carotenoids in augmenting the tissue antioxidative status in broiler chicken. Compared to unsupplemented controls there were significantly decreased peroxidation level in the tested tissues (liver, heart ventricles and brain) of spirulina/carotenoid supplemented broilers. Upasani *et al.* (2001) studied the effect of spirulina supplementation in rats in which lipid peroxidation was induced by administration of lead through deionised water. Supplementation of spirulina considerably reduced the levels of peroxidation products in the liver, lung and kidney. The finding of the present experiment is congruent with the observations of the earlier studies mentioned above.

5.6. EFFECT OF DIETARY SUPPLEMENTATION OF *SPIRULINA PLATENSIS* ON FERTILITY PARAMETERS IN EGG TYPE MALE CHICKEN

Reproduction in male is influenced by many factors and nutrition claims an important role in it. Ross and Domini (1990) have reported the effect of *Spirulina platensis* in female Japanese quails. These authors observed statistically significant increase in fertility (% fertile eggs), hatchability of fertile eggs, and hatch of all eggs set in spirulina-fed quails. All the same, male fertility studies have not been published till date. Superior nutritive value of many algae including spirulina raises the possibility that spirulina could bring about desirable changes in the reproductive performance of livestock too. However, investigations directly addressing this issue fall short hitherto.

5.6.1. Gross Evaluation Scores for Semen Samples

The appearance of semen sample depends upon the concentration of spermatozoa and the volume of seminal fluid. Highly concentrated semen samples will be thicker and hence will appear creamy (Roberts, 1986). Scores are usually given based on the visual examination of semen immediately after collection (as mentioned in section 3.8.1). Average semen evaluation scores in this experiment were found to be higher for alga-fed birds in G II group over the controls in all the three collections (table 10a).

5.6.2. Semen Volume

Semen volume collected was significantly higher for G II birds in group (spirulina-fed) (table 10b). In the purview of the current experiment, the quantity and quality of semen can be considered to be governed by two sets of factors. Firstly, there are direct influence of the diet, management and normal physiological process regulating the activity of the testes and reproductive tract in forming semen. Secondly, there are factors determining the degree of response of a male to the massage technique of semen collection and the amount of semen that is extracted from the erected ejaculatory ducts. Thirdly, feeding, rearing and housing can influence semen production, temperature, season, age, frequency of semen collection and breed also influences the semen volume and sperm concentration (Maule, 1962). As a result, the observed values can have wide variations (Parker, 1949; Lake, 1957; Sturkie, 1976).

Semen volume was found to be significantly higher for G II group (spirulina-fed) during the entire collection periods. Parker and Mc Spadden (1943) studied the effect of nutrition on seminal volume and showed that when food was restricted seminal volume decreased. Deficiencies of vitamins A and E in the diet have shown to affect spermatogenesis and semen production adversely

(Lorenz, 1959). Increased semen volume in birds belonging to spirulina-fed group could be due to their better plane of nutrition.

5.6.3. Mass Activity

No significant difference in mass activity of the semen samples could be appreciated between two groups (table 10a). The mass activity and motility estimation can be used to detect gross differences in the semen quality, but they have limited value in determining small fertility differences. There is no clear evidence that mass activity can be closely related with fertility and studies have yielded negative or indefinite results in bulls (Cupps *et al.*, 1953.)

5.6.4. Motility

Motility of the spirulina treated groups was found to be higher in G II group in all the months of study (table 10a). The motility estimation, just like mass activity can only be used to detect gross differences in the semen quality. Higher motility of the semen samples from spirulina treated birds indicated superior fertility for the same.

5.6.5. Methylene Blue Reduction Time

Methylene blue reduction time depends on the dehydrogenase activity, which in turn depends on the overall metabolic activity of the semen (Hafez and Hafez, 1993). An assessment is made from the time required by a given semen sample to decolourise a solution of methylene blue under standard conditions of incubation. Jenichen *et al.* (1965) have demonstrated a significant correlation between methylene blue reduction time and conception rate of semen. In the present study the methylene blue reduction time of G II group (spirulina-fed) was significantly lower (table 4-10) which could indicate a better metabolic activity in semen samples from G II group and hence better quality.

5.6.6. Estimation of Live and Dead Sperms

Spirulina-treated group showed a significantly higher level of live sperms in differential staining during all the three collections (table 4-10). The fact that this staining method does not differentiate motile from non-motile spermatozoa but differentiate only living from dead spermatozoa was a matter of debate and hence the validity of this test in assessing the fertility of the animal (Lasley, 1961). But studies have indicated a positive correlation between the live and motile spermatozoa in the various samples examined (Bane, 1972). The present results also indicated a positive correlation between the percentage of live sperms and the percentage of motile sperms.

Higher percentage of live sperms in spirulina-treated birds underlines the protective role of spirulina against sperm damage while storage. The deferent duct is the site where semen is stored prior to ejaculation, particularly the recepataculum of the deferent duct. Sperm is liable to many forms of oxidative damage while on storage (Hafez and Hafez 1993). Since the spirulina is having a protective role against the scavenging free radicals a higher percentage of live sperms in spirulina-supplemented birds show that sperms are better protected against damage from the free radicals. Hypoosmolarity swelling test also showed significantly higher level of live sperms in spirulina treated group (G II) vide table 10b

5.6.7. Resistance of Spermatozoa against Heat and Cold Shock

No significant difference was found between the heat shock resistance times of semen samples from the two groups (table 10c). This test is done to evaluate the ability of the spermatozoa against an induced heat shock and to assess the keeping quality and fertilizing capacity of semen. No significant difference was found between the percentages of motile spermatozoa in the

semen samples of birds from two groups after the induction of cold shock (table 10c).

The present study aimed to find the effects of supplementation of spirulina in Austra-white male chicken has not only reinstated some of the already established therapeutic properties of spirulina but also helped in identifying its effect on many new aspects. The fertility effect, however, needs further confirmation, preferably by insemination trials. Of course, only subcellular and molecular level studies can resolve the intrigues in this area.

Many properties of spirulina like growth stimulating, haematopoietic, hypolipidaemic, hypocholestraemic and antioxidant effects as re-established in species could further open up new commercial/research avenues in poultry industry. Above all, the results from this study could point to the potential role of this emerging feed supplement in enhancing fertility in male birds, possibly forming the first report of its kind.

Summary

6. SUMMARY

The present study was undertaken with an objective of evaluating the influence of dietary supplementation of *Spirulina platensis* on certain haematological, biochemical and fertility parameters of Austra-white male chicken. Thirty-two numbers of eight-week-old Austra-white male chicken were procured from Kerala Agricultural University poultry farm and reared under standard management conditions in battery cages. The birds were selected randomly, weighed, wing banded and divided into two groups, G I and G II comprising of sixteen birds in each. Birds of group G I (control) were solely fed with standard layer ration while birds of G II group were fed on layer ration with supplemented with *Spirulina platensis* (powder) at the rate of 2.5% of feed. Both rations were made isocaloric and isonitrogenous. Body weight of the birds was taken at monthly intervals up to thirty-two weeks of age. Blood was collected with heparin at monthly intervals from third to eighth month of age. Analysis of haematological parameters such as total erythrocyte count (TEC), haemoglobin content (Hb), volume of packed red cells (VPRC), erythrocyte sedimentation rate (ESR) and total leukocyte count (TLC) were done. Erythrocytic indices were calculated from the estimated values of TEC, Hb and VPRC. Plasma was used for biochemical analysis viz., plasma protein profile (total protein, albumin, globulin, and calculation of albumin globulin (A: G) ratio), plasma lipid profile (total cholesterol, total triglycerides, and total lipids), concentration of blood urea nitrogen (BUN) and plasma level of bilirubin at monthly intervals from third to eighth month of age. Plasma antioxidant activity was assessed by estimating the activity of blood catalase and plasma lipid peroxidation level. Semen was collected from the birds at twenty-fourth, twenty-eighth and thirty-second weeks of age. Semen evaluation was done by observing the motility, mass activity, semen volume, methylene blue reduction time (MBRT), differential staining and hypo osmolarity swelling, and heat and cold shock resistance tests in the collected semen samples. Birds were sacrificed at thirty-two weeks of age and the

relative organ weight of liver, spleen, pancreas and testes was estimated. Lipid peroxidation levels of homogenized tissue samples of liver, spleen, pancreas and testes were also estimated. Statistical analyses of the data were done using split anova technique.

Spirulina-fed birds (G II group) had a significantly higher body weight through out the experimental period compared to birds of G I group (controls). Relative organ weight of spleen, pancreas and testes were also significantly higher for the birds fed on *Spirulina platensis* (G II group) while the relative weight of liver did not show any significant difference between the two groups. The results are suggestive of the usefulness of spirulina as a nutritious feed supplement for chicken. Birds of G II group receiving spirulina supplementation showed a significantly higher TEC, Hb, VPRC and TLC values during the entire period of study. ESR was significantly lower for G II group cockerels during this period. Values of MCV were significantly higher for chicken of G I group (controls). While the MCH values did not show any significant difference between the two groups the MCHC values were significantly higher for chicken receiving *Spirulina platensis* (G II group). Thus, it is indicated that there was a more marked increase in total erythrocyte count with moderate increase in the haemoglobin content of spirulina-fed cockerels over the control birds. Statistically insignificant increase in MCH values in spirulina fed birds of group II over the controls is a result of this discrepancy. The discrepancy might be due to the fact that spirulina brought forth its erythropoietic effect in the cockerels heavily by stimulating mitotic activity of the haemopoietic stem cells or by increasing the average life span of red blood cells.

Plasma concentration of protein, albumin and globulin were significantly higher for spirulina fed G II group than the controls (G I group). The spirulina-fed group also showed increased levels of BUN and bilirubin levels in plasma when compared to the control birds. The results are indicative of the high protein content of spirulina with superior digestibility and biological value. The plasma

lipid profile revealed a low level of total lipid and cholesterol of spirulina-fed birds in the G II group while the levels of triglyceride was significantly higher than the control birds. Results confirmed that spirulina exhibited strong hypolipidaemic and hypocholesteraemic properties while the increased triglyceride level may be due to the lipolytic effect.

Estimation of blood catalase activity indicated that spirulina-fed birds of G II group showed a high activity than control group. Plasma peroxidation level was significantly lower for spirulina-fed birds when compared to the controls. Estimation of peroxidation levels in homogenised samples of liver and pancreas also showed a significantly lower peroxidation level in spirulina-fed group. The results indicated that spirulina bestowed profound antioxidant protection to its subjects.

Gross evaluation of the semen samples indicated that semen from spirulina-fed group were thicker and had higher motility when compared to the control birds (G I group). Semen volume was also significantly higher for spirulina-fed birds. Assessment of the metabolic activity of the sperms by methylene blue reduction time showed that semen from spirulina-fed birds took significantly lesser time for reduction compared to semen samples of control birds. An estimate about the percentage of live sperms in the semen samples was done by differential staining and hypoosmolarity swelling test. Significantly higher percentage of live sperms in birds of G II group (spirulina-fed) was observed when compared to the birds of G I group. Heat and cold shock resistance test did not reveal any significant difference between the semen samples from the two groups. The results of the semen evaluation studies indicated an improved fertility status in birds of spirulina-fed G II group when compared to G I group which in turn is suggestive of fertility augmenting property of spirulina.

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EFFECT OF BLUE GREEN ALGA (*Spirulina platensis*) ON HAEMATOLOGICAL, BIOCHEMICAL AND FERTILITY PARAMETERS OF EGG TYPE MALE CHICKEN

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ABSTRACT

Enhancement of total production without compromising aspects of wholesomeness and subject /consumer /environmental safety of the produce forms the new challenge of Indian poultry industry. Since nutrition is the single largest external factor affecting performance of the stock and accounting lion share of the input cost, nutritional manipulations have widely been used to take up this challenge. Especially in the wake of set backs from potential residual hazards of anabolic steroids used as growth promoting feed additives, use of certain blue green algae as dietary supplement offers more innocuous organic alternatives, for being safe to the subject, consumers and environment.

Owing to their success in human health with anabolic and therapeutic effects cyanobacteria, the blue green algae, are being tried in livestock/poultry production as well. Spirulina is rich in protein, vitamins and provitamins such as carotenoids, and minerals. Protein from spirulina is believed to be highly digestible due to the lack of a cell wall; it contains 18 amino acids, major ones being lysine, arginine, threonine, methionine and phenylalanine. High levels of arginine is normally believed to be insulinogenic, indirectly through stimulation of growth hormone secretion

The present study forms part of an exploration of growth promoting and fertility augmenting aspects of one of the popular species of blue green algae, *Spirulina platensis*, envisaging evaluation of its influence on haematological, biochemical and fertility parameters of Austra-white male chicken, when used as a dietary supplement. Thirty-two numbers of eight-week-old Austra-white male chickens were reared under standard management conditions in battery cages in Department of Physiology, College Of Veterinary and Animal Sciences. The birds were selected randomly and divided into two groups, G I (control) and G II (spirulina supplemented) comprising of sixteen birds in each. Birds of group G I

were solely fed with standard layer ration and formed the controls in the experiment while birds of G II group were fed layer ration with dietary supplementation of *Spirulina platensis* at the level of 2.5% of feed. Both rations were made isocaloric and isonitrogenous. Feed and water were provided to birds *ad libitum.*

Body weight of the birds was taken at monthly intervals from initial period (eight week of age) to thirty-two weeks of age. Blood was collected at monthly intervals from third to eighth months of age. Estimation of haematological parameters comprised total erythrocyte count (TEC), haemoglobin content (Hb), volume of packed red cells (VPRC), erythrocyte sedimentation rate (ESR) and total leukocyte count (TLC). Erythrocytic indices were calculated from the estimated values of TEC, Hb and VPRC. Biochemical profile of plasma included estimation of total protein, albumin, globulin, albumin globulin (A: G) ratio (protein profile), cholesterol, triglycerides, total lipids (lipid profile), blood urea nitrogen (BUN) and bilirubin at monthly intervals from third to eighth month. Plasma antioxidant status was assessed by estimating catalase enzyme activity and lipid peroxidation level. Semen was collected at twenty-fourth, twenty-eighth and thirty-second weeks of age. Semen evaluation was done by observing the motility, mass activity, semen volume, methylene blue reduction time (MBRT), percentage of live sperms (differential staining and hypo osmolarity swelling) and heat and cold shock resistance tests in the collected semen samples. Birds were sacrificed at thirty-two weeks of age and the relative organ weight of liver, spleen, pancreas and testes was estimated. Lipid peroxidation level of homogenized samples of liver, spleen, pancreas and testes were also estimated. The data were statistically analysed using appropriate tests.

Body weight of birds fed with spirulina (G II group) was significantly higher when compared to control (G I group). Relative organ weight also showed a significantly higher relative weight for spleen, pancreas and testes. The birds of G II group (*Spirulina platensis*- fed) showed a significantly higher values of TEC, Hb concentration, VPRC and TLC compared to control (G I group) birds.

The value of ESR was significantly lower for the spirulina fed group. Plasma protein profile indicated a significant higher concentration of total protein, albumin and globulin levels in spirulina-fed group. Plasma lipid profile had a low total lipid and cholesterol level in spirulina fed birds of G II group when compared to the controls in G I group. However, the triglyceride levels was significantly higher for spirulina fed birds (G II group). Plasma concentrations of BUN and bilirubin were also significantly higher for G II group. The results indicated a strong haematopoietic effect of spirulina and its usefulness as a protein rich nutritional supplement for poultry.

Plasma antioxidant activity was significantly higher for G II group in the sense that the spirulina fed group showed an increased blood catalase activity and a decreased plasma lipid peroxidation levels. Estimation of peroxidation level in homogenised samples of liver and testes also had a significantly lower peroxidation level in birds of G II group. Results underlined the strong antioxidant capacity of spirulina.

Semen evaluation studies revealed a significantly higher semen volume, motility, and percentage of live sperms in semen samples from birds of G II group (spirulina fed) when compared to G I group. Semen samples from G II group also showed a significantly lower methylene blue reduction time compared to G I group. Heat and cold shock resistance test failed to show any significant difference between the two groups. Results indicated the fertility augmenting property of spirulina.

The present study is consistent with earlier reports of growth promoting and erythropoietic effects of spirulina. Free radical scavenging effect of this species of alga has caused a marked increase in the antioxidant status of the subjects, congruent with earlier findings. Additionally, this is the first publication to report indications of a fertility augmenting effect of this alga in male birds. However, confirmation of the latter effect needs further investigation, preferably with insemination studies.