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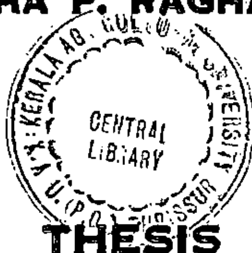
EVALUATION OF ANTISTRESS AND GROWTH PROMOTING EFFECT OF ASWAGANDHA *(Withania somnifera)* IN BROILER CHICKEN

By

REKHA P. RAGHAVAN



THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

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Department of Veterinary Physiology

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

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2009

DECLARATION

I hereby declare that the thesis, entitled **"EVALUATION OF ANTISTRESS AND GROWTH PROMOTING EFFECT OF ASWAGANDHA (*Withania somnifera*) IN BROILER CHICKEN"** is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award 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 **"EVALUATION OF ANTISTRESS AND GROWTH PROMOTING EFFECT OF ASWAGANDHA (*Withania somnifera*) IN BROILER CHICKEN"** is a record of research work done independently by **REKHA P. RAGHAVAN**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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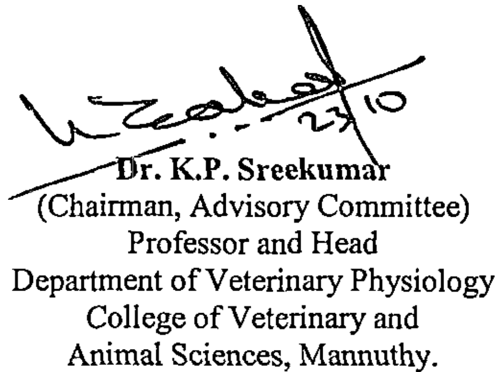

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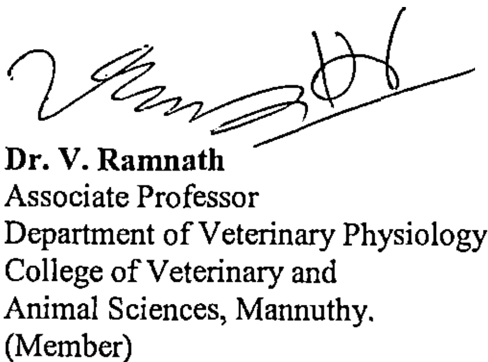

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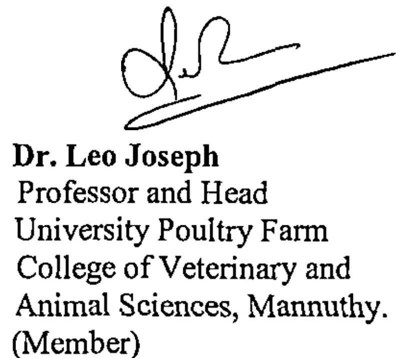

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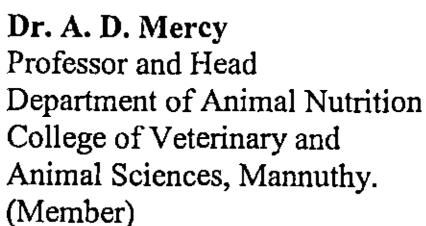

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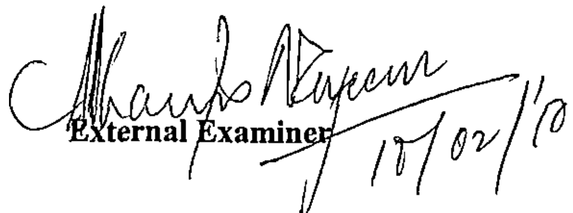

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REKHA P. RAGHAVAN

TO

BELOVED MEMORY OF MY FATHER

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Introduction

1. INTRODUCTION

Herbal growth promoters are being used for improving growth and production in animals. There are many herbal medicine used as growth promoter in poultry industry.

Withania somnifera known as Aswagandha (Indian ginseng) is an important herb used in indigenous medicinal system. *W. somnifera* is a small woddy shrub of *solonaecia* species that grows about two feet in height. It is found in India, Africa and Meditaranian countries. The root of the plant is the main portion used therapeutically.

The major active constituents of roots are steroidal alkaloids and steroidal lactones under the class of constituents called withanolides. *W. somnifera* contains a bitter alkaloid somniferin, having hypnotic property. Root and bitter leaves are used as hypnotic agents. At present 12 alkaloids, 35 withanoliodes and several sitoindosides have been isolated from the plant. The pharmacological action has been attributed mainly to Withanolide D. The Withaferin A is a therapeutically active alkaloid present in leaves and roots and has vast range of application in the treatment of various disorders, toxicities and also for improving growth and production.

The alcoholic extract of *W. somnifera* has been found to reduce stress in rats (Dadkar *et al.*, 1987). In Ayurveda, *W. somnifera* is considered as a rasayana herb, which works on a nonspecific basis to increase health and longevity. This herb is also considered as adaptogen as it normalizes physiological function, through the hypothalamo-adenohypophyseal axis (HPA axis) and neuroendocrine system (Agarwal, 1994).

W. somnifera is proved to be a general tonic, antistress, hepatoprotective, haematinic, growth promoter and antioxidant in human practice (Pandae and Vijayakumar, 1994). The alcoholic root extract of the *W. somnifera* contain an

active compound Withaferin A which showed significant antitumor and radiosensitizing effect in experimental tumors *invivo* with out noticeable systemic toxicity (Devi, 1996). The alkaloid Withaferin A is reported to have antiarthritic, antibiotic, antimicrobial, antimitotic, viricide and antitumor activity (Lindner, 1996).

The root and berry of the plant are also used in herbal medicine. They are used as adaptogen for patients with nervous exhaustion and debility, and as immunostimulant in patients with low white blood cell count. It exerts a positive influence on the endocrine system and enhanced thyroxine (T_4) concentration in mice (Pandae and Kar, 1998).

There are good number of studies regarding the effect of *W. somnifera* in human beings and animals but only less in poultry especially under the influence of stress. The present study was undertaken in broiler chicken with the following objectives

(1) To evaluate the effect of *W. somnifera* as an antistress agent.

(2) To evaluate the effect of *W. somnifera* as a growth promoter.

Review of Literature

2. REVIEW OF LITERATURE

Broiler production plays a major role in food security for the rapidly increasing Indian human population. Compared to other domestic animals, broiler chickens are more susceptible to changing environmental conditions. Stress depresses the feed intake, weight gain and increases the mortality rate among broilers. A possible approach to counteracting the negative effect of stress among chicken could be through the supplementation of antistress agents. Various antistress agents are used to reduce the stress and improve growth and production in poultry industry.

2.1 EFFECT OF *WITHANIA SOMNIFERA* AS A GROWTH PROMOTER

Grandhi *et al.* (1994) investigated about the comparative pharmacological effects of *Withania somnifera* and Ginseng in mice. The aqueous suspension of roots of *W. somnifera* and Ginseng were tested for antistress activity and anabolic activity. *W. somnifera* treated group showed increase in body weight and antistress activity compared to Ginseng treated group.

Wheeler (1994) reported that Zeetress® (*W. somnifera* is one of the ingredients) treated broiler birds exhibited increase in body weight gain and improvement in feed conversion ratio and reduction in mortality when compared to untreated ones.

Anand and Kuttan (1995) reported that an increase in size of spleen in *W. somnifera* treated mice enhanced the immune system by stimulating the spleen cells.

Maiti *et al.* (1995) concluded that supplementation of Zeetress® in drinking water at a dose rate of 1g / 100 birds daily for 10 days significantly improved the egg production, egg quality and body weight with little difference in feed consumption in layers.

Aphale *et al.* (1998) found that a combination of Ginseng and *W. somnifera* fed to rats diet caused significant increase in feed consumption, body weight, liver weight and improved haematological parameters. Total weight gain was significantly more than control group. There was no change in weight of spleen and kidney between experimental and control group.

Mishra and Singh (2000) studied the effect of feeding root powder of *W. somnifera* on growth, feed consumption, efficiency of feed conversion and mortality rate in broiler chicks. G1, G2 and G3 were supplemented with 0.5 per cent, 1 per cent and 2 per cent *W. somnifera* root powder in the feed and G4 served as control. The differences between groups for weekly body weight gain from day old chicks to eight weeks of age were found non significant but at third week significant differences between groups were noticed. The weight gain in group G1 was better than control group. The feed consumption in control group was slightly higher than treated group. Feed conversion efficiency in G1 and G2 was better than G3 and control. The cumulative feed conversion efficiency in treatment groups was better than control group.

Rajeshwari *et al.* (2001) reported the effect of Zeetress® on broiler performance. The live weight and feed conversion was significantly better in Zeetress® administered chicks. Zeetress® helped to minimize stress and had a positive effect on live weight, feed conversion and general resistance.

Randae *et al.* (2002 a) indicated that supplementation of Immuplus (standardised extracts of *W. somnifera, Ocimum sanctum, Tinospora cordifolia* and *Emblica officinale)* @ 250 mg/50 birds from 1st to 4 th week of age through drinking water, caused improvement in feed conversion ratio. The birds supplemented with Immuplus had lower feed consumption compared to control group.

Samarth *et al.* (2002) studied the effect of *W. somnifera* root powder on the performance of broilers chicken. T1 was maintained as control while, T2 was provided with root powder of *W. somnifera* 0.5 per cent as feed mix. There was no significant difference in average live body weight between T2 and T1. Feed consumption, feed conversion efficiency of treated group was 631.92 and 2.10 and control group was 611.47 g and 2.22 respectively. The average body weight and dressed weight of T2 and T1 was 1707.50 g and 1458.33 g, 1552.50 g and 1241.66 g respectively. T2 showed significantly higher live weight and dressing weight.

Reddy (2003) concluded that dietary supplementation of Zeetress® caused no significant variation in body weight and weight gain of birds in floor space reduced group compared to those kept on optimal floor space.

Narayananswamy and Santhosh Kumar (2004) reported the beneficial effect of Geriforte® which has *W. somnifera* is one of the ingredients, in commercial broiler chicken in summer stress. The birds supplemented with Geriforte® showed an increase in body weight, feed conversion ratio and faster growth rate than control group.

Akotkar *et al.* (2007) studied the effect of supplementation of 0.5, 0.75, 1.0 and 1.25 per cent of *W. somnifera* root powder to broilers. Feed consumption was lowered in broilers fed with 1.25 per cent *W. somnifera*. Feed efficiency varied significantly between treatments. The poorest feed efficiency was seen in control group, which was improved with 0.5 per cent *W. somnifera* supplemented group and better feed efficiency was found in 0.75 per cent *W. somnifera* supplemented group.

Pedulwar *et al.* (2007) studied the effect of dietary supplementation of *W. somnifera* on broiler chicken. The control group was supplemented with standard broiler diet while, the remaining groups were fed *W. somnifera* at 0.5 and 1 per cent levels up to six weeks of age. Live weight and cumulative weight

gain was significantly increased in 0.5 per cent *W. somnifera* supplemented groups. Feed conversion and feed consumption was less in 0.5 and 1 per cent *W. somnifera* supplemented group compared to control group.

Jadhav *et al.* (2008) reported the effect of supplementation of *W. somnifera* as antistress agents on growth performance and immune status of broilers during hot weather. T0 group was supplemented with a standard broiler diet, whereas T1 was provided with 1 per cent *W. somnifera* root powder. The body weight and weight gain increased significantly in supplemented groups. Average feed consumption and cumulative feed consumption per bird was not significantly different among the groups, whereas the mean feed conversion ratio significantly improved with supplementation of *W. somnifera.*

Shisodiya *et al.* (2008) studied the effect of *W. somnifera* in the performance of chicken broilers. *W. somnifera* supplemented at the rate of 0.05 per cent in feed, showed significantly increased live body weight, weekly body weight gain, feed conversion ratio and feed consumption compared to control group. Feed consumption in control group was higher than supplemented group.

Javed *et al.* (2009) evaluated the effect of aqueous extract of plant mixtures *Zingiber officinale, Carum apticum, Withania somnifera, Trigonella Foenum Graecum, Silybum marianum, Allium sativum and Berberis lyceum,* on the growth performance of broiler chicks. Total numbers of chicks were divided into four groups (A, B, C and D), each having 20 chicks. Aqueous extract of these plants was mixed at the rate of 5, 10 and 15 ml/l with water and was offered to group B, C and D, respectively, while, group A served as a control. Mean weight gain, dressing percentage, breast weight and leg weight were significantly higher in group C with lower FCR (Feed Conversion Ratio) while, mean feed intake was significantly higher in control group.

2.2 EFFECT OF *WITHANIA SOMNIFERA* AS AN ANTISTRESS AGENT

Singh *et al.* (1982) reported that the rejuvenating herbal drug i.e. *W. somnifera* had adaptogenic property. It prevented gastric ulcers induced chemically or by stress in rats. The drug prevented increase in adrenal weight, decreased ascorbic acid and cortisol content of adrenal gland during stress.

Chatterjee (1994a) reported that Zeetress® inhibited the undesirable response to stress, when administrated in rats up to 100 mg/kg body weight for 30 days. Immobilization stress affects the cardiovascular system of adult male rats. Significant alterations in E.C.G. pattern and blood pressure were noticed in rats exposed to the stressor. Zeetress® a polyherbal antistress and adaptogen, protected the animals from stress-induced alterations in E.C.G. and blood pressure profile.

Chatterjee (1994b) indicated that stress induced leukocytosis was reduced in mice treated with Streszee® (one ingredient being *W. somnifera*)

Wheeler (1994) reported that the use of herbal supplement *W. somnifera,* had antistress and immunomodulatory activities. It reduced the effect of stress in intensively housed chicken. It decreased six percent in feed conversion ratio following treatment. Birds treated with herb had a lower visceral fat content and all these were attributed to the antistress activity of the herbal supplement.

Bhattacharya and Ghosal (1994) reported that Zeetress® did not exert any discernible antistress activity on a single acute administration, but attenuated stress following sub chronic administration (5-10 mg /kg) orally for 7 days. Zeetress® reduced the incidence of stress induced gastric ulcer and level of plasma corticosterone, adrenal ascorbic acid and adrenal gland weight in rats.

Zeetress,® a poly herbal antistress agent contained the extracts of *W. somnifera* and *ocimum sactum* as major constituents was shown to ameliorate different undesirable effects of stress in chicken (Wheeler, 1994 and Das, 1994).

Kaur *et al.* (2001) studied the effect of administration of *W. somnifera* water suspension (360mg / kg body weight.) and compound X (20 mg / kg body weight) a biologically active compound of *W. somnifera*, in multiple stressed rats. The rats treated with either aqueous suspension or compound X could withstand the multiple stress of cold -hypoxia- restraint (CHR) better than control rats and compound X showed better result than the aqueous suspension *W. somnifera.* The drugs treated animal could withstand the stress much better than control group.

Bhattacharya and Muruganandam (2003) reported the adaptogenic activity of a standardized extract of *W. somnifera* roots in a rat model of chronic stress (CS). The stress procedure was mild, unpredictable foot shock, administered once daily for 21 days to adult male Wistar rats. Chronic stress expressed by significant hyperglycemia, glucose intolerance, increased plasma corticosterone levels, gastric ulcerations, male sexual dysfunction, cognitive deficits and immunosuppression. These CS induced perturbations were attenuated by *W. somnifera* (25 and 50 mg/kg po) and by *Panex ginseng* (100 mg/kg po), administered 1 hour before foot shock for 21 days. The results indicated that *W. somnifera* and *Panex ginseng* have significant antistress adaptogenic activities.

2.3 EFFECT OF *WITHANIA SOMNIFERA* ON HAEMATOLOGICAL PARAMETERS

Ziauddin *et al.* (1996) studied the haematological effect *W. somnifera* in mice, where myelosuppression was induced by cyclophosphamide, azathioprin and prednisolone therapy. There was a significant increase in haemoglobin concentration, platelet count, RBC and WBC count, and body weight in *W. somnifera* treated mice compared with control mice.

Aphale *et al.* (1998) reported that combination of Ginseng and *W. somnifera* fed to rats caused improvement in haematopoiesis. There was an increase in haemoglobin and RBC count in treated group.

Davis and Kuttan (2000) reported that administration of an extract from the powdered root of *W. somnifera* stimulated immunological activity in mice. Treatment of *W. somnifera* root extract (20 mg/dose/animal; i.p.) enhanced the total white blood cell count on 10th day of administration.

Rajeshwari *et al.* (2001) reported the effect of Zeetress® on broiler performance. The heterophil lymphocyte ratio was normal in Zeetress administrated group than unsupplemented group.

Reddy (2003) reported the effect of dietary supplementation of Zeetress® and ascorbic acid in floor space reduced broiler chicken. Dietary supplementation of Zeetress® and ascorbic acid in floor space reduced groups and feed restricted groups elevated the lymphocyte counts, suppressed heterophil and basophil counts, H: L ratio and considerably improved the mitogen induced lymphoblastogenic response in both the groups at sixth and eighth week of age.

Samarth *et al.* (2003) reported the effect of *W. somnifera* on hemato-biochemical profile of broilers. T1 was designated as the control while, T2 was treated with root powder from *W. somnifera* at 5 per cent in the feed mix. There was increased haemoglobin concentration and haematocrit in T2 compared to T1.

Dhote *et al.* (2005) reported the effect of Immuplus in chicken. Chicks given Immuplus, at the rate of 25 mg / body weight caused significant increase in total leukocyte and absolute leukocyte count than untreated group.

Akotkar *et al.* (2007) studied the effect of supplementation of 0.5 per cent, 0.75 per cent, 1 per cent and 1.25 per cent of *W. somnifera* root powder to broiler chicken. Haematological studies revealed significant increase in haemoglobin concentration, packed cell volume, in *W. somnifera* treated birds than control.

Wajari *et al.* (2007) observed the effect of supplementation of root powder of *W. somifera* @ 5 g/kg feed and fruit powder of *Emblica officialis* 500 mg/ kg feed to broiler chicken. *W. somnifera* supplemented group showed significant

increase in platelet count, packed cell volume, total leukocyte count and total erythrocyte count in chicken compared to control group. There was no significant difference in Hb concentration in treated group.

Oyagbemi *et al.* (2008) reported that prolonged administration of Stresroak® (*W. somifera* as an ingredient) in grower cockerels improved the hematological parameters in chicken compared to control group. A significant increase in total RBC count, WBC count, and mean corpuscular haemoglobin concentration were observed at 30 days post administration. Heterophil lymphocyte ratio (H:L) was reduced in birds treated with Stresroak®. The non significant increase in PCV, RBC, Hb and MCH in treated group at 60 days post administration.

Daisy *et al.* (2008) reported the effect of *W. somnifera* on haemato-biochemical profile of broilers. Group I was kept as control. Groups II and III were given *W. somnifera* root extract (WRE) at 100 and 300 mg/kg body weight, respectively in the drinking water, groups IV and V were given *W. somnifera* extract leaf (WLE) at 100 and 300 mg/kg body weight, in drinking water. The values of Hb and PCV after feeding of WRE at a dose of 300 mg/kg/day were 14.55 g/dl, 34.00 per cent respectively. The values were significantly higher than control group. (WRE) at 300 mg/kg body weight significantly increased the haematological parameters. WRE at a dose of 100 mg/kg body weight could not significantly alter the value. WLE did not change the haematological parameters compared to control group.

Singh *et al.* (2009) narrated the haematological alterations in broilers supplemented with different herbal formulations during summer, rainy and winter season. The birds were supplemented with amla and turmeric powder 5 g / kg of feed, Zeetress® 250 mg/ 500 ml drinking water and Zist 250 mg/ kg of feed to different experimental birds. There was marked improvement in haemoglobin, lymphocyte count and decrease in heterophil count in birds supplemented with herbal formulation and Zeetress®.

2.4 EFFECT OF *WITHANIA SOMNIFERA* ON BLOOD BIOCHEMICAL PARAMETERS

Geetha (1993) observed that stress induced hypercholesterolemia in control rats was significantly attenuated by Stresszee® (a product similar to Zeetress®) at a dose of l0 mg / kg, indicating antiatherogenic action with faster mobilization of glucose in stressed animals.

Sudhir and Budhiraja (1992) reported the protective effect of Withaferin A (alkaloid of *W. somniferea*) against CCl_4 induced hepatotoxicity at a dose rate of 10 mg / kg in rats. Rats treated with 3 doses of CCl_4 alone developed significant hepatic damage (cirrhosis of liver) as observed from significant rise in the levels of SGOT, SGPT and serum alkaline phosphatase. Treatment of rats with 10 mg/kg dose of Withaferin A or hydrocortisone 10 mg/kg protected the liver significantly as deduced from the reduction in SGOT, SGPT and serum alkaline phosphtase.

Geetha (1993) and Kamath (1994) revealed that stress induced elevated glucose, urea, total protein, AST, ALT, ALP and LDH activities were significantly decreased when treated with Stresszee® in rats.

Chatterjee (1994) reported that Zeetress® did not have adverse effect on body weight, feed consumption, haemopoietic system and biochemical parameters when administrated to rats upto 100 mg/kg body weight in feed for 30 days.

Andallu and Radhika (2000) reported the hypoglycaemia effect of *W. somnifera* root powder, where treated group showed significant decrease in blood glucose compared to that of oral hypoglycemia drug (metformin). Hypoglycemic, diuretic, and hypocholesterolemic effects of *W. somnifera* root were assessed in human subjects, in which six type 2 diabetes mellitus subjects and six mildly hypercholesterolemic subjects were treated with *W. somnifera* powder extract for 30 days. A decrease in blood glucose comparable to that of an oral hypoglycemic drug was observed.

Samarth *et al.* (2003) reported the effect of *W. somnifera* on hemato-biochemical profile of broilers. T1 was designated as the control while, T2 was treated with root powder from *W. somnifera* at 5 per cent in the feed mix. There were significant increase in blood glucose, total serum protein, albumin and globulin values in T2 compared to T1. The blood glucose, total protein, albumin, globulin in T1 and T2 were 221.55 mg/dl and 228.70 mg/dl, 3.44 g/dl and 4.51 g/dl, 1.8 g/dl and 2.02 g/dl, 1.41 g/dl to 1.41 g/dl respectively.

Visavadiya and Narasimhacharya (2007) reported that, *W. somnifera* when added to diet @ 0.75 and 1.5 g / day in hypercholestermic rats caused significant decrease in total lipids, cholesterol and triglycerides in plasma. This was due to significant increase in HMG COA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase) activity and bile acid content of liver in the animals. It also reduced the lipid peroxidation in hypercholesteremic animals.

Daisy *et al.* (2008) reported the effect of *W. somnifera* on haemato-biochemical profile of broilers. Group I was kept as control. Groups II and III were given *W. somnifera* root extract (WRE) at 100 and 300 mg/kg body weight, respectively in the drinking water, groups IV and V were given *W. somnifera* leaf extract (WLE) at 100 and 300 mg/kg body weight, in drinking water. A significant increase in the serum protein concentration of groups III (3.94 g/dl) and group V (3.87 g/dl) was observed compared to control when treated with WRE and WLE @ 300mg/kg/ day.

Oyagbemi *et al.* (2008) studied the effect of Stresroak® on grower cockerels. The plasma levels of total protein, globulin, and albumin increased dose dependently both at 30 and 60 days post Stresroak® administration. The plasma levels of ALP and AST were significantly lowered while, non significant changes were observed for plasma levels of ALT and GGT at 30 days and 60 days post Stresroak® administration.

Vidyarthi *et al.* (2008) studied the effect of herbal growth promoters on performance of broiler chicken, where T1 was control group and T2 supplemented with Zeetress® @ 0.05 g/bird daily. The serum glucose level was higher (168.90 mg/dl) in T1 group compared to T2 (149.13 mg/dl) group. The birds supplemented with herbal growth promoters showed increased body weight compared to control group.

Avnish *et al.* (2009) studied the effects of combination of Shilajit (*Asphaltum punjabianum*) and *W. somnifera* on fasting blood sugar and lipid profile in patients. Herbal capsules containing aqueous extract of *W. somnifera* 250 mg and pure Shilajit extract 250 mg were prepared as investigational drug. It was observed that in diabetic patients the herbal drugs significantly reduced fasting blood sugar, low density lipids, very low density lipoprotein, total cholesterol and high density lipoprotein ratio.

Udayakumar *et al.* (2009) reported the hypoglycaemic and hypolipidaemic effect of *W. somnifera* root extract (WSREt) and leaf extract (WSLEt) on alloxan induced diabetic rats. Haemoglobin, total protein, albumin globulin ratio (A: G), tissue protein and glycogen were significantly decreased in alloxan induced diabetic rats. The levels of serum total cholesterol, phospholipids, very low density lipoprotein and low density lipoprotein were significantly increased in diabetic rats when compared to those of normal control rats. Treatment of the diabetic rats with WSREt and WSLEt restored the changes of the above parameters to their normal level after eight weeks of treatment, indicating that WSREt and WSLEt possess hypoglycemic and hypolipidaemic activities in alloxan-induced diabetes mellitus (DM) rats.

2.5 EFFECT OF *WITHANIA SOMNIFERA* AS AN ANTIOXIDANT

Panda and Kar (1997) evaluated the free radical scavenging activity of *W. somnifera* root powder in mice.The effects of aswagandha root powder administrated @ 0.7 and 1.4 g/kg body weight/ day for 15 and 30 days was

investigated on lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase activities in mice. *W. somnifera* treated with 30 days produced a significant decreased in LPO and an increased in SOD and catalase, while 15 days treatment produced no significant change. The values observed in LPO, SOD and catalase of control and 0.7 g and 1.4 g treatment group were 0.48 nm of MDA, 5.92 Units/mg of protein, 55.19 μm of H_2O_2 decomposed /min/mg protein, 0.35 nm of MDA, 7.56 Units/mg of protein, 67.15 μm of H_2O_2 decomposed /min/mg protein, 0.25 nm of MDA, 9.21 Units/mg of protein, and 71.32 μm of H_2O_2 decomposed /min/mg protein respectively.

Dhuley (1998) evaluated the effect of *W. somniferea* on lipid peroxidation in stress induced animals. Elevation of lipid peroxidation (LPO) was observed in rabbits and mice after intravenous administration of 0.2 μg /kg of lipopolysaccharide (LPS: from *Klebsiella pneumoniae*) and 100 μg /kg of peptidoglycan (PGN: from *Staphylococcus aureus*) respectively. The peak was reached immediately after PGN and 2-6 h after LPS administration. Simultaneous oral administration of *W. somniferea* (100 mg/kg) prevented the rise in LPO in rabbits and mice.

Bhattacharya *et al.* (2001) reported the antioxidant effect of *W. somnifera.* Glycowithanolides (WSG), an alkaloid of *W. somnifera* administered 10, 20 and 50 mg / kg body weight in mice induced a dose related reversal of chronic foot shock stress effects. WSG normalize the augmented superoxide dismutase and lipid peroxidase activities and enhanced the activities of catalase and glutathione peroxidase.

Kaur *et al.* (2004) reported that leaf extract of *W. somnifera* had significant antiproliferative activity in human tumorigenic cells but did not impart any protection against oxidative damage caused by high glucose and hydrogen peroxide to human tumor cell.

Kumar *et al.* (2005 a) evaluated the effect of Zeetress® and vitamin C supplementation on salinomycin toxicity in broiler chicks. Both antioxidants protected the bird against salinomycin toxicity in birds. Erythrocyte glutathione peroxidase, glutathione reductase and catalase were elevated and glutathione concentration was reduced significantly in salinomycin intoxicated birds. Salinomycin toxicity was mediated by free radical generation and depletion of glutathione level due to oxidative damage. There was no significant change in the values observed in control and salinomycin intoxicated birds, indicating that Zeetress® prevented the broiler against salinomycin toxicity.

Harikrishnan *et al.* (2008) investigated the influence of *W. somnifera* root powder on the levels of circulatory ammonia, urea, lipid peroxidation products such as TBARS (thiobarbituric acid and reactive substances), HP (hydroperoxides) and liver marker enzymes such as AST (aspartate transaminase), ALT (alanine transaminase) and ALP (alkaline phosphatase), for its hepatoprotective effect in ammonium chloride induced hyperammonemia in rats . Ammonium chloride treated rats showed a significant increase in the levels of circulatory ammonia, urea, AST, ALT, ALP, TBARS and HP. These changes were significantly decreased in rats treated with *W. somnifera* root powder. The *W. somnifera* offered hepatoprotection by influencing the levels of lipid peroxidation products and liver markers in experimental hyperammonemia. This was due to the presence of alkaloids, withanolids and flavonoids, normalizing the levels of urea and urea related compounds, through its free radical scavenging antioxidant properties.

2.6 EFFECT OF *WITHANIA SOMNIFERA* AS AN IMMUNOMODULATOR

Das and Chatterjee (1994) observed that administration of Streszee® in rats resulted in an increase of lymphocyte population dynamics in differential leukocyte count and potentiated cellular and humoral components of immune system. Streszee® was a safe product even at ten times the higher dose level (100 mg / kg body weight) on daily administration 30 days. There was a significant

increase in lymphocyte, plasma globulin, weight of adrenal gland and eosinophils when Streszee® was administrated at the dose of 100 mg / kg body weight.

Ziauddin *et al.* (1996) studied the immmunomodulatory effect of *W. somnifera* in mice, where myelosuppression was induced by cyclophosphamide, azathioprin and prednisolone therapy. *W. somnifera* prevented myelosuppression in mice treated with the three immunosuppressive drugs.

Agarwal *et al.* (1999) reported the immmunomodulatory effect of *W. somnifera* extract WST and WS2 in experimental immune inflammation in mice. Immunomodulatory effect was arrested by IgE mediated anaphylaxis and delayed type hypersensitivity. In the delayed type hypersensitivity reaction (DTH) the modulatory effect was assessed as potentiation or suppression of the reaction. Potentiation of DTH reaction was observed in animals treated with cyclophosphamide at a dose of 20 mg/kg body weight, WST at a dose of 1000 mg/kg and WS2 at a dose of 300 mg/kg. Cyclophosphamide induced potentiation of reaction was suppressed in animals treated with WST and WS2. A significant increase in white blood cell count and platelet count was observed in animal treated with WST and WS2.

Davis and Kuttan (2000) reported that administration of root extract of *W. somnifera* stimulated the immunological activity at a dose rate of 20 mg/ dose; i.p of mice. *W. somnifera* treated group enhanced antibody titre and the number of antibody producing cells in spleen. The maximum number of plaque forming cell in *W. somnifera* treated group ($985/10^6$ spleen cells) was observed on 4th day after administration while, control animal had only a maximum of 310 PFC /10^6 spleen cells.

Deka *et al.* (2002) reported the immunomodulatory effect of Stresroak®and levamisole on maternal immunity against Newcastle disease. The Stresroak® treatment to breeder white leghorn following vaccination against

Newcastle disease induced better humoral immune response. The antibody titre was highest on the day of hatching in progeny chickens of Stresroak® and Levamisole treated birds compared to untreated birds.

Kumar *et al.* (2002) studied the immunomodulatory effect of Zeetress ® on immunosuppressive action caused by aflatoxin in NDV (New castle disease virus) and salmonella vaccinated birds. Zeetress ® supplemented group showed significant increase in antibody titre when supplemented at a dose of 5 g/1000 birds in drinking water.

Randae *et al.* (2002 b) indicated that the supplementation of Immuplus improved the feed conversion ratio of birds exposed to stress. It maintained the high level of protection in birds against Infectious bursal disease (IBD) and New castle disease virus by raising the antibody titre, under stressful condition.

Diwany *et al.* (2004) reported the immunoprotection of botanical drugs in cancer chemotherapy. Treatment of ascitic sarcoma bearing mice with total extract of *W. somnifera* and *Tinospora cardifolia* protected cyclophosphamide induced myelosuppression in mice. It caused significant increase in white cell count and haemagglutinating and haemolytic antibody titre.

Khan *et al.* (2004) studied the proliferation of T lymphocyte and Th1 cytokines by *W. somnifera* in stressed mice. Oral administration of chemically standardized aqueous fraction of *W. somnifera* at the graded dose 25, 50, 100 and 200 mg/kg p.o caused significant increased in the stress induced depleted T cell population and increased expression of Th1 cytokines in the chemically stressed mice.

Manoharan *et al.* (2004) studied the effect of polyherbal ingredient Stresroak®on day old chick quality by feeding parent stock with Stresroak ® in feed mix @ 2 kg / ton of feed. The result showed that chick quality score of the *W. somnifera* supplemented group was significantly different from control group. The average chick quality score of the control group ranged between 88.46 and

90.06. In the Stresroak® supplemented groups the value ranged from 93.43 and 94.94. The transfer of maternal antibody against Ranikhet disease in the Stresroak® supplemented group showed significant difference compared to control group.

Dhote *et al.* (2005) studied the effect of Immuplus, an herbal immunomodulator on the paraspecific immunity in developing stages of chicks. One group was continuously administered with Immuplus at the rate of 25 mg/kg body weight in drinking water and the other group was kept as control. All the chicks were vaccinated against New Castle disease (NCD) and infectious bursal disease (IBD) as per the prescribed schedule. TLC of treated group was 27.26, 27.78 and 28.07 $x10^3/\mu l$ and corresponding values for control group was 25.73, 26.21, and 26.88 $x10^3/\mu l$ respectively at 55, 50 and 60 days. The absolute lymphocyte count was also increased in treated group compared to control group.

Padmavathi *et al.* (2005) studied the effect of *W. somnifera* in the carcinogen induced fore stomach and skin tumorigenecis in Swiss albino mouse. *W. somnifera* inhibited benzo (a) pyrene induced fore stomach papillomagenesis and 7, 12 dimethylbenzanthracene induced skin papillomagenecis in mice.

Hindustani and Singh (2006) studied the immunomodulatory effect of Zeetress® in IBD vaccinated broiler chicken. Group I received infectious bursal disease (IBD) vaccine at 0.05 ml/bird intraocularly and Zeetress® powder added to the drinking water at 5 mg/bird from day 3 to 28 and 10 mg/bird from day 29 to 42. Group II received only interplus IBD vaccine, while Group III served as control. At five days of age, all groups were given F strain of Newcastle disease virus vaccine intraocularly. Serum samples were collected at 13 days of age and at 21, 28, 35 and 42 days of age (post-IBD). Serum samples were examined for IBD antibody titre. Zeestress® expressed immunopotentiating effect in vaccinated birds, with better immune response to Newcastle disease virus vaccine, improvement in live weight gain and feed conversion efficiency. The herbal

preparation also exhibited enhancing effect on the immune response to IBD vaccine.

Senthilnathan *et al.* (2006) studied the immunomodulatory effect of *W. somnifera* extract on benzo (a) pyrene induced experimental lung cancer in albino mice. Benzo (a) pyrene induced experimental lung cancer animal were treated with *W. somnifera* extract at the dose rate of 400 mg/kg body weight. The 30 days treatment with *W. somnifera* extract altered the level of immunoglobulin. The carcinogen affects the immune system and the toxic side effect of the immune system were reversed and controlled by *W. somnifera.*

Kolte *et al.* (2007) observed the immunomodulating effect of *W. somnifera* and Tinospora cordifolia in broiler birds. T1 was control, while other four T2, T3, T4 and T5 were immunosupressed with cyclophosphamide @ 150 mg/kg body weight. There was significant improvement in the values Hb, PCV and TLC of birds treated with *W. somnifera at* the rate of 2 g/kg in the feed.

Jadhav *et al.* (2008) reported the effect of supplementation of *W. somnifera* as antistress agents on growth performance and immune status of broilers during hot weather. T0 group was supplemented with a standard broiler diet, whereas T1 was provided with 1 per cent *W. somnifera* root powder. The inclusion of 1 per cent supplementation of *W. somnifera* resulted in an insignificant increase in HI titre over the control group.

2.7 EFFECT OF STRESS ON CERTAIN PARAMETERS IN BROILERS

2.7.1 Effect of Stress on Production Parameters

Tomhave and Seeger (1945) found that reduction in floor space resulted in lower body weight, increased mortality and poor feed consumption in broilers. However Siegel and Coles (1958) reported that there were no significant difference in the body weight and feed conversion efficiency in broilers reared at various floor levels ranging from 0.5 to 1.25 sq ft / bird.

Bolton *et al.* (1972) reported that decrease in space allowance in broilers from 0.093 to 0.047 m^2/ bird was accompanied by reduced final live weight and feed consumption and improved feed conversion efficiency.

Bhargava *et al.* (1975) reported that caged broilers when provided the floor space of 0.70 sq ft / bird weighed significantly less than those reared at densities of 0.50 and 0.65 sq ft/ bird.

Zoog (1980) reported that there was decreased growth rate with increased meat production in broilers per unit floor space by increasing the stocking density.

Pesti and Howarth (1983) reported that body weight gain during first week in broiler chicken kept at 697 cm^2 /bird was significantly lower than those kept at 348 cm^2 / bird, although the chicks at 697 cm^2 ate more feed indicating poor feed conversion efficiency.

Donkoh (1989) conducted an experiment to elucidate the influence of four constant ambient temperatures (20°, 25°, 30° and 35°C) on the performance and physiological reactions of male commercial broiler chicks from 3 to 7 weeks of age. Exposure of broiler chickens to the 20°, 25°, 30° and 35°C treatments showed highly significant depression in growth rate, feed intake and efficiency of feed utilization, and a significant increase in water consumption for the 30° and 35°C groups.

Al-Batshan and Hussein (1990) observed that cyclic high temperatures reduced the body weight, feed intake, carcass weight and increased feed conversion ratio in broiler chicken.

Kuan *et al.* (1990) reported that when broilers were provided a floor space of 0.095, 0.071, 0.051 and 0.048 m^2/ bird; with increasing stocking density the feed consumption reduced and improved feed conversion without effecting growth rate up to six week. After six weeks, birds at lowest stocking rate had highest average daily gain (39.2 g) and daily feed consumption (127.0 g) and

poorest feed conversion (3.3 g feed/g gains). Fowls at highest stocking density had lower average daily gain (32.2 g) and feed consumption (90.6 g) and they concluded that stress associated with overcrowding had reduced the performance of broilers.

Cravener *et al.* (1992) reported that there was no effect of population density treatments on feed conversion at sixth or seventh week of age in broilers. Birds housed at 0.07, 0.09 and 0.11 m^2 /bird had significantly higher body weight and carcass weight than the birds housed at 0.05 m^2/bird, indicating stress at higher stocking densities.

Gill and Sharma (1992) opined that the stocking density did not significantly affect the six week body weight gain in both deep litter and on wooden slat floors reared chicken. At eighth week of age broilers at floor space of 1 sq.ft/ bird were better than 0.75 sq ft/ bird, and the birds on slat floor were significantly heavier and consumed more feed at both the stocking densities than birds reared on deep litter system. There was no significant difference in feed efficiency of birds either between floor systems on stocking densities. The birds kept on deep litter at 1 sq ft /bird density did not differ significantly in their body weight but the birds having 0.75 sq ft floor space had significantly lower eviscerated weight. At eight weeks of age the birds on 1 sq ft /bird had high carcass weight, eviscerated and giblet weight.

Narayanankutty and Ramakrishnan (1992) reported that broilers reared in California cage 60x45x45 cm at densities three, four, and five birds per cage showed higher body weight, carcass yield, and lower mortality with better feed efficiency in birds of five birds/ cage. The overall mean body weights of broilers at eight weeks of age were 1338.89 g, 1312.50 g and 1243.33 g respectively for groups with 3, 4 and 5 birds per cage. The feed efficiency was 3.38, 3.06 and 2.89 respectively. The mean feed consumption was highest in groups reared in cages with three birds and lowest in cases with five birds.

Puvadolpirod and Taxon (2000) concluded that experimental induction of stress by continuous administration of ACTH (8 IU/day i.v for seven consecutive days) in chicken resulted in decreased body weight, feed intake and reduced the relative weight of bursa, thymus and spleen. They also observed that body weight did not return to normal value one week after cessation of ACTH administration.

Feddes *et al.* (2002) demonstrated the effect of stocking density in broiler performance. The stocking densities of 23.8, 17.9, 14.3, and 11.9 birds /m^2 corresponded to 260, 195, 156 and 130 birds /pen respectively. Birds grown at 23.8 birds /m^2 had lower body weight and carcass weight compared to birds grown at 14.3 birds /m^2. Stocking density had no effect on mortality, breast meat yield, carcass grade, incidence of scratches, or carcass quality. The high yield per unit area and good carcass quality could be achieved at the increased stocking density when ventilation rates were adequate.

Vecerek *et al.* (2002) reported that high temperature on 16 th day of fattening had decreased the weight of broiler chicken. The increase in temperature caused depression the growth of chicken.

Mashaly *et al.* (2004) reported the effect of heat stress on production parameters and immune response in laying hens. Body weight, feed consumption egg production, egg weight and egg shell thicknesses decreased in heat stressed group compared to control group.

Shivakumar *et al.* (2004) reported the performance of broiler at different floor space. The birds were reared at floor space of 0.50 (T1), 0.75 (T2), 1.0 (T3) and 1.25 (T4) sq ft / bird. Birds reared on 1.25 sq ft / bird showed significant increase in body weight, feed consumption and feed efficiency. The bodyweight of T1, T2, T3 and T4 were 1561.87 g, 1552.00 g, 1545.60 g, and 1596.70 g respectively. The feed consumption and feed efficiency of T1, T2, T3 and T4 were 3241 g, 2956 g, 2540 g, 2782 g and 2.132, 1.963, 1.695 and 1.794 respectively. Better feed efficiency was observed in1.00 sq ft / bird followed by

1.25 sq ft / bird and 0.75 sq ft / bird. Feed consumption was higher in 0.5 sq ft/bird.

Bilgili and Hess (1995) reported that the placement density influenced broiler carcass grade and meat yields. Male and female broilers were housed during warm weather at varying placement densities to determine the influence of density on carcass grade and yield. Males were reared to 49 days of age at densities of 0.8, 0.9, or 1.0 ft. ^{2}/bird; females were raised at densities of 0.65, 0.75, or 0.91 ft. ^{2}/bird to 42 days. Live performances of males were influenced by increasing placement density with negative influences on 49-day body weight and feed conversion. Females showed a similar non significant trend in body weight. The percentage of carcasses was reduced at the highest density in females. The placement density had impact in carcass quality, yield, and live performance.

Dozier *et al.* (2005) examined responses of male broilers during a 49-day production cycle to 4 placement densities in 2 trials. In each trial, 1,488 male chicks were randomly placed into 32 floor pens to simulate final densities of 30, 35, 40 and 45 kg of BW/m^2 of floor space. Growth rate and nutrient utilization were similar among the treatments from 1 to 32 day of age. From 1 to 49 day, body weight gain and feed consumption were adversely affected by increasing the placement density from 30 to 45 kg of body weight/m^2 of floor space.

Ipek and Sahan (2006) reported the effect of cold stress on broiler performance. The chicks were divided into control and cold stress group. Body weight gained upto three weeks were significantly decreased in cold stress group.

Mejo (2006) reported that there was no significant difference in weight of spleen and adrenal gland in heat stressed birds compared to control bird.

Onbasilar and Aksoy (2004) studied the effect of stocking density on performance and stress in broilers. The birds were reared into stocking density groups 11.9 and 17.5 broilers /m^2. Broilers reared in 11.9 broilers/m^{2}showed

significant decrease in initial weight, body weight gain and higher percentage of gizzard weight.

Samale *et al.* (2008) studied the performance of broilers reared under recommended management condition and compared it with those reared under various stress conditions. Stress of overcrowding and poor litter conditions affected body weight gains during latter weeks of age from 4th week. Feed intake was significantly affected by overcrowding from 4th week onwards, and poor litter condition from 4th week onwards as compared to control birds. Feed conversion ratio (FCR) under stress of poor feeding was significantly inferior from 2nd week onwards. FCR was not affected significantly by poor litter conditions.

Nayanatara *et al.* (2009) reported that chronic stress caused a significant increase in the weight of the adrenal gland in Wistar strain adult albino rats.

2.7.2 Effect of Stress in Haematological Parameters

Donkoh (1989) elucidated the influence of four constant ambient temperatures (20°, 25°, 30° and 35°C) on the physiological reactions of male commercial broiler chicks from 3 to 7 weeks of age. Changes in physiological status, such as increased rectal temperatures, decreased concentration of red blood cells, haemoglobin, and haematocrit were observed in birds housed in the higher temperature (30° and 35°C) environments.

Emre *et al.* (1991) revealed that population density range of 10, 14, 18 and 20 chicks /m^2 did not affect the volume of packed red cell (VPRC) and haemoglobin (Hb) value in broilers of 40 to 49 days of age.

Flora and Ranjini (2000) reported the effect of temperature on haematological parameters in guinea fowl during summer season and winter season. There was no significant difference between sexes in serum Ca, P, Hb, VPRC, total leukocyte count (TLC), total erythrocyte count (TEC), lymphocyte and eosinophil level during summer and winter..

Vecerek *et al.* (2002) studied the influence of environmental temperature on production haematological and biochemical index in broilers. High environmental temperature increased the value of haemogloin and reduced the amount of leukocyte in chicken blood. The values of Hb in normal and stressed birds were 80.22 to 91. 41 g/l and 76.45 to 84.12 g/l, while TLC were 14.6 to 19.00 and 19.45 to 21.31 G/l respectively.

Bedaoova *et al.* (2003) reported the haematological profile of broilers under acute and chronic heat stress at 30 ± 1^0C for 24 hours. Acute heat stress at 42 days in male and female broiler showed a significant decrease in haematocrit value and haemoglobin level following long term exposure to high temperature. Decrease in lymphocyte and increase in heterophil lymphocyte ratio (H: L) were seen in both sexes.

Reddy (2003) reported that stocking density of broilers 22 birds /m^2 did not produce any significant change in Hb or VPRC values in six and eight week old broilers. Erythrocytopenia was observed in feed restricted broilers, where as floor space reduction (33 per cent) had no effect on total the TEC. According to him, neither feed restriction nor the floor space reduction could induce any change in the values of mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) value and mean corpuscular volume (MCV).

Mashaly *et al.* (2004) observed that leukopenia in laying hens subjected to heat stress. The total red blood cell count and antibody production was significantly inhibited in heat stressed group. The immune response decreased in birds subjected to heat stress.

Bedanova *et al.* (2006) studied the effect of reduction of floor space during crating on haematological indices in broilers. The birds which had reduction in floor space 105 cm ^{2}/ kg, compared with 115 cm^2/ kg showed a significant increase in haemoglobin level, MCV, MCH, MCHC and decrease in erythrocyte count.

Mejo (2006) could not observe any significant difference in Hb and VPRC values in five and ten days heat stressed birds compared to control birds. Heat stress brought about severe leukocytopenia associated with significant reduction in absolute lymphocyte count and relative increase in heterophil count.

Aengwanich (2007) reported the effect of high environmental temperature on MCH, MCHC and MCV of broiler chicken. The MCV and MCHC of the chickens maintained at 38±2°C was significantly higher than that of chickens maintained at 26±2°C, while the MCH value decreased.

Bedanova *et al.* (2007) studied the haematological parameters in acute heat stress due to shackling in broilers. When compared with control group, shackled group exhibit decrease in haemoglobin level, erythrocyte count and mean corpuscular haemoglobin concentration. The value of normal group and stressed group ranged from 128.78 g/dl and 100.60 g/dl, 3.38 x10^6/µl and 2.35 x 10^6/µl, 50.76 pg and 37.78 pg respectively.

Karthiayini and Philomina (2008) studied the effect of overcrowding stress on haematological parameters of broiler chicken. The control groups were provided with floor space of 696 cm^2/bird and experimental group the floor space of 348 cm^2/bird. The haematological parameters such as TEC, Hb, PCV, MCV, MCHC, MCH, and TLC in normal and stressed groups were 2.88x10^6/µl and 2.90x10^6/µl, 8.54 g/dl and 8.83 g/dl, 27.42 per cent and 27.33 per cent, 95.37 fl and 94.70 fl, 31.46 pg and 32.80 pg, 29.67 per cent and 30.55 per cent, 25.67 x10^3/µl and 25.83x10^3/µl respectively. The values were not affected by the applied stress of overcrowding in both season and significantly high heterophil count and lower lymphocyte count was noticed in stressed birds.

2.7.2.1 Heterophil Lymphocyte Ratio (H:L)

Mc Farlane *et al.* (1989) reported that H: L increased with heat stress in broiler chicks. Elevation of H: L was observed with increased stocking density.

Kuan *et al.* (1990) reported that increased stocking density produced stress indicated by elevated H: L from the four week onwards.

Gross and Siegel (1983) observed that H:L of about 0.2, 0.5 and 0.8 characterised by low, optimum and high levels of stress respectively. They suggested that in birds H: L was a good measure of long term stress in chicken (hours / weeks).

Atlan *et al.* (1999) reported the effect of heat stress on some blood parameters in broilers. Broilers exposed to 39^0C significantly increased the rectal temperature, and H: L and decreased monocytes and lymphocyte proportion. H: L was increased from 0.25 to 0.43. Acute heat stress did not affect the haematocrit value.

Reddy (2003) observed H :L of normal chicken and stressed chicken at fourth to eighth week of age ranged from 0.42 to 0.41 and 0.42 to 0.52. There was no significant difference in H:L in floor space reduced group.

Spinu *et al.* (2003) reported that there was no difference in H:L between different stocking densities in broiler breeders in summer.

Onbasilar and Aksoy (2004) investigated the effect of cage floor and cage density on stress parameters of laying hens. Hens were allocated in 1, 3 and 5 in each cage to obtain different cage density of 1968, 656 and 393.8 cm^2 floor area / hens. The ratio of heterophil to lymphocyte of the group having 5 hens was higher than 3 hens. The group with 5 hen per cage had higher H:L than other groups with 3 hen and 1 hen.

Zulkifli and Sti Nor Azah (2004) reported that the heterophil to lymphocyte ratio was a reliable indicator of avian stress. Broiler exposed to heat stress in summer showed an increase in heterophil and decrease in lymphocyte, which lead to increase in H:L.

Bedanova *et al.* (2007) studied the haematological parameters in acute heat stress in broilers due to shackling. When compared with control group, shackled group showed significant increase in heterophil count, and H:.L.

2.7.3 Effect of Stress in certain Biochemical Parameters

2.7.3.1 Serum Glucose

Siegel (1961) reported that stress increased the circulating glucocorticoides, which in turn elevated the blood glucose concentration in young chicken.

Sawnhey *et al.* (1986) reported that stress induced hyperglycemia was generally observed as a consequence of increased adrenaline and possible suppression of insulin like activity in man.

Donkoh (1989) reported hyperglycemia in broilers at high environmental (30-35^{0}C) stress. There was increased blood glucose concentration and decreased thyroid gland in stressed birds.

Vecerek *et al.* (2002) reported that high environmental temperature increased the level of plasma glucose and cholesterol in broiler chicken. Plasma glucose and cholesterol in control group were 13.90 to 16.45 mmol/l and 2.11 to 3.19 mmol/l and experimental group was 10.59 to 14.75 mmol/l and 1.81 to 2.95mmol/l respectively. Plasma glucose significantly increased in experimental group.

Reddy (2003) did not observe any significant change in plasma glucose value in 42 and 56 days old broilers maintained at a stocking density of 22 birds /m^2 for a period of 45 days. Plasma glucose of normal birds ranged from 235.84mg/dl to 233.60 mg/dl and stressed birds ranged from 233.80 mg/dl to 233.16 mg/dl respectively.

Onbasilar and Aksoy (2004) investigated the effect of cage floor and cage density on stress parameters of laying hens. Hens were allocated in different cage density of 1968, 656 and 393.8 cm^2 floor area / hen with one, three and five hens respectively. The blood glucose level was higher in hens with floor space of 393.8 cm^2 floor areas / hen. The group with five hen 393.8 cm^2 floor spaces had increased blood glucose level.

Karthiayini (2007) studied the effect of overcrowding stress on plasma glucose of broiler chicken. The control groups were provided with floor space of 696 cm^2/bird and experimental group the floor space of 348 cm^2/bird. The plasma glucose in normal and stressed group ranged from 200.91 to 248.43 mg/dl and 206.65 to 271.04 mg/dl respectively. The plasma glucose was not significantly affected in stressed group.

Nayanatara *et al.* (2009) reported that chronic stress in Wistar albino rats caused significant increase in blood glucose and cholesterol, and also tissue malondialdehyde (MDA) in liver and kidneys.

2.7.3.2 Serum Total Protein, Albumin, Globulin, Albumin Globulin Ratio and C-reactive protein

Ward and Peterson (1973) reported that broiler chicken subjected to chronic heat stress (33 to 35^0C for 4 weeks) had significantly lower plasma protein concentration than those reared at 18 to 22 ^{0}C.

Polonis (1982) reported that the serum albumin concentration increased in five to eight weeks old chicks when exposed to 12 to 14 ^{0}C and 24 to 26 ^{0}C.

Donkoh (1989) observed that reduction in total plasma protein in broilers housed in the higher temperature (30° and 35°C) compared to 20^0C and 25^0C.

Deyhim *et al.* (1995) found that heat stress reduced the serum albumin concentration in broiler chicken.

Belay and Teeter (1996) observed a decrease in the serum protein and albumin concentrations in seven week old broilers subjected to cycling temperature of 24 to 35 °C.

Fenghua *et al.*(1997) observed a lowered A:G in 350 days old laying hen exposed to 34.5^0C for 10 to 14 days, when compared to those exposed to 26^0C.

Berrong and Washburn (1998) showed that plasma protein level of broiler chicks exposed to 38 ° C was significantly lower than birds reared at 21^0C.

Reddy (2003) could not observe any significant difference in the levels of plasma albumin, globulin, A:G in broilers under overcrowding stress (429 cm^2/bird) or feed restriction stress. The value of albumin, globulin and A : G of normal groups at fourth to eighth week of age ranged from 2.25 g/dl to 2.59 g/dl, 3.03 g/dl to 2.94 g/dl and 0.75 g/dl to 0.89 g/dl respectively. In stressed group it ranged from 2.42 g/dl to 2.77 g/dl, 2.87 to 2.95 g/dl and 0.85 g/dl to 0.95 g/dl respectively.

Kumar *et al.* (2005 b) studied the effect of serum CRP(C reactive protein) level during experimental mycotoxicosis and its amelioration using antioxidants in broiler chickens. They found that the CRP level in the serum of aflatoxin feed birds increased on day 14 to 42. They observed a significant decrease in the serum CRP levels in birds continually fed with mycotoxins and antioxidants supplements.

Mejo (2006) reported that cockerels subjected to five and ten days of heat stress produced no significant difference in total protein, albumin and globulin concentration compared to non heat stressed birds. She also studied the effect of heat stress on serum C reactive protein level in gooseberry (GB) and Indian gallnut (IGN) treated cockerels. The control birds showed a value of 0.72 mg/dl,

while the heat stressed birds treated with GB and GN exhibited a value of 1.44 mg/dl on second day of heat exposure but untreated birds exhibited the value of 5.76 mg/dl on sixth day of heat exposure.

Karthiayini (2007) studied the effect of overcrowding stress on biochemical parameters of broiler chicken. The control groups were provided with floor space of 696 cm^2/bird and experimental group the floor space of 348 cm^2/bird. The total protein, albumin, globulin, albumin globulin ratio in normal and experimental birds were 5.13 g/dl and 5.03 g/dl, 2.28 g/dl and 2.32 g/dl, 2.86 g/dl and 2.83 g/dl, 0.80 g/dl and 0.88 g/dl respectively. Overcrowding did not significantly affect the above parameters. The globulin level was decreased by overcrowding stress.

2.7.3.3 Serum Total Cholesterol

Donkoh (1989) noted hypercholestremia in broilers exposed to heat and cold stress.

Cetin and Tuncel (1995) concluded that stress symptoms include increase in concentration of plasma total cholesterol in broiler chicks of six week and eight week of age, when stocking density increased to 22 chicks/ m^2 or more.

Reddy (2003) did not observed any significant change in plasma total cholesterol value in 42 and 56 days old broilers maintained at a stocking density of 22 birds /m^2 for a period of 45 days. Plasma cholesterol of normal birds ranged from136.60 mg/dl to 169.95 mg/dl and stressed birds ranged from 147.80 mg/dl to 165.39 mg/dl.

Karthiayini (2007) studied the effect of overcrowding stress on total cholesterol of broiler chicken. The control groups were provided with floor space of 696 cm^2/bird and experimental group the floor space of 348 cm^2/bird. The total cholesterol in normal and stressed group ranged from 110.26 mg/dl to 158.03

mg/dl and 105.24 to 163.35 mg/dl respectively. The total cholesterol was not significantly affected by overcrowding stress.

2.7.3.4 Serum Enzymes

Karthiayini (2007) studied the effect of overcrowding stress on plasma ALT of broiler chicken. The control groups were provided with floor space of 696 cm^2/bird and experimental group the floor space of 348 cm^2/bird. The plasma ALT in normal and stressed group ranged from 34.42 to 42.67 U/l and 34.17 to 44.83 U/l respectively. The plasma ALT was not significantly affected in stressed group.

Nayanatara *et al.* (2009) investigated the changes in selected biochemical and lipid parameters following exposure to chronic unpredictable stressors for 10 days. Wistar strain adult albino rats were divided into two groups as non stressed group and stressed group. The stressed groups were exposed to 10 days of chronic unpredictable stress. A significant increase serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were observed in the stressed group, when compared to the non stressed group.

2.7.4 Effect of Stress on certain Antioxidant parameters

2.7.4.1 Tissue Lipid Peroxidation and Reduced Glutathione

Sahin *et al.* (2001) conducted an experiment on protective role of supplemental vitamin E on lipid peroxidation, in broilers reared under heat stress. Increased supplementation of vitamin E linearly increased serum and liver vitamins A and E and decreased MDA concentrations. Dietary Vitamin E had significant effect on minerals, where the serum copper concentration increased.

Mahmoud and Edens (2003) reported that broiler chicken fed with organic selenium as sel-plex®, selenised yeast had elevated glutathione peroxidase (GPX) activity in blood and liver after heat stress. This was due to less induction of heat

shock protein70 (hsp70) in organic selenium fed chicken. Organic selenium fed chicken was less affected by mild heat stress than inorganic selenium fed chicken.

Yuvaraj *et al.* (2003) reported the tissue antioxidative status of broiler chicken fed with spirulina and natural carotenoids. Broilers were supplemented with one per cent dried spirulina powder in the feed. The GSH content of liver was significantly higher and lipid peroxidation was lower in supplemented birds than control group. The GSH and LPO activity in normal and supplemented birds were 88.6 and 105.5 nmol/ml homogenate and 5.44 and 3.72 nmol/ml homogenate respectively.

Reddy *et al.* (2004) reported the antioxidant and hypolipidemic effect of spirulina and natural carotenoids in broiler chicken. Birds were supplemented with one per cent spirulina powder in the feed. The serum GSH activity significantly increased and lipid peroxidation reduced in birds supplemented with spirulina powder compared to control group. The GSH and LPO activity of control and supplemented birds are 50.7 and 55.0 nmol/ml, 3.65 and 2.42 nmol/ml respectively.

Maini *et al.* (2007) conducted comparative study of antioxidants Vitamin E and Amla electrolyte in broilers. They observed that in normal broiler chicken of three and five week of age the GSH levels were 1.33 and 0.97 mmol / l respectively, while the LPO level were 12.44 and 9.24 nmol respectively. The birds supplemented with Vitamin E (200 mg/kg feed) and amla electrolyte (1 g /kg of feed), GSH were 1.79 and 1.30, mmol / l, 1.45 and 2.09 mmol / l respectively, while LPO 4.26 and 1.35 nmol / l, 1.22 and 2.89 nmol / l respectively. All the values were significantly different from experimental group.

Ramnath *et al.* (2007) conducted a study on four week old local strain male chicken, to investigate the effect of Brahma Rasayana (BR) supplementation on concentrations of certain oxidative stress markers associated with heat stress. Liver reduced glutathione levels were found to be significantly lower in heat

stressed (HST) untreated chickens, when compared with treated groups. Glutathione peroxidase in heat stressed and heat stressed BR supplemented group was 9.23 U/mg of protein and 15.54 U/mg of protein respectively. Thus BR supplementation during HST brings about enhanced action of enzymatic and non-enzymatic antioxidants, which nullified the undesired side effects of free radicals that were generated during HST.

Ramnath and Rekha (2009) conducted a study on four week old local strain male chicken, to investigate the effect of an ayurvedic supplementation of Brahma Rasayana on antioxidant status of cold stressed male chicken. The study was conducted in three groups. GSH and LPO of liver of normal birds, cold stressed untreated and cold stressed BR treated birds were 1.53, 2.04 and 2.02 nmol/mg of protein and 0.50, 1.01 and 0.45 nmol of MDA formed /mg of protein respectively. There was significant decrease in lipid peroxidation in cold stressed BR treated birds than cold stressed birds. No significant difference was observed between cold stressed and cold stressed BR treated birds in tissue GSH level.

Tatliseven *et al.* (2009) reported the effect of selenium and Vitamin C supplementation on lipid peroxidation of broiler at cold environment. The birds fed with high energy diet were compared with Vitamin C and selenium supplemented group. Malondialdehyde levels of liver and catalase activities of liver of control group were higher than supplemented group. The GSH activities of liver were not found significantly different between control and supplemented group. MDA and catalase activity of group supplemented with Se and Vitamin C were decreased.

2.7.5 Effect of Stress in Immunological Parameters

Subba Rao and Glick (1977) studied the effect of cold exposure on the immune response of chicken. They observed that birds exposed to 32.2^{0}C significantly depressed the antibody titre against SRBC.

Henken *et al.* (1982) investigated the effect of environmental temperature on the humoral immune response of pullets following injection of SRBC. They found a significant increase in total haemagglutinin anti SRBC antibody titre from day 5 after immunization in pullet maintained at 40^0C compared to antibody titre in pullets maintained at 25^0C.

Pardue *et al.* (1985) studied the role of ascorbic acid in male chickens exposed to high temperature (38^0C). The anti-sheep red blood cell (anti-SRBC) haemagglutinin level after challenging with SRBC (1 ml, 10^5 cells, iv) suggested that heat mediated immunosuppression was ameliorated by ascorbic acid supplementation.

Sharma and Ray (1997) studied the effect of herbal formulation septilin, as immune modulator on immune response in mice. Oral administration of septilin 500mg / kg along with immunosuppressive drugs (prednisolone) 4mg/ kg enhanced both primary and secondary immune response in mice treated with sheep red blood cell (SRBC). There was a significant increase in body weight, spleen/ body weight ratio in septilin treated group and decrease in prednisolone in the treated group.

Guo and Liu (1997) concluded that heat stress significantly reduce the feed intake, body weight gain, feed conversion efficiency and inhibited the normal development of lymphoid organs and impaired immunological competence in broilers.

Onbasilar and Aksoy (2004) investigated the effect of cage floor and cage density on stress parameters of laying hens. Increase in the cage density decreased the antibody titre to SRBC. Antibody titre was higher in cage with one hen than five hens.

Mejo (2006) studied the effect of heat stress on immune response in cockerels were treated with SRBC. The haemagglutination titre of normal birds and heat stressed birds evaluated on one and five day after immunization. Heat

stressed showed a low profile of humoral immune response indicated by low HA titre.

Kenan (2008) investigated the effect of stocking density on the heterophil to lymphocyte ratio, blood corticosterone concentration, and immune response in broilers during summer. Stocking density had no significant effect on H: L ratio, blood corticosterol concentration and immune response in broiler chicken, when the birds were housed at density of 15, 20, and 25 birds /m^2.

Zahraa and Al-Ghandi (2008) studied the effect of heat stress on immune responses in broiler chickens reared in closed system. The birds were exposed to 40°C/4 hrs/day for ten days. The heat stress led to significant decrease in antibodies (IgG and IgM) levels.

2.7.6 Gastrointestinal enzymes

Plimer and Rosedale (1921) studied the distribution of enzyme in the alimentary canal of the chicken. Lipase was presented only in the pancreas of the chicken.

Laws and Moore (1963) investigated the pancreatic and small intestinal lipase activity in chicks. The lipase activity with an emulsified triglyceride as substrate was confined almost entirely to the pancreatic extracts, whereas esterase activity with a water soluble substrate was confined almost entirely to extracts of the small intestine.

Sadanandan (1968) detected the presence of high proteolytic activity of the proventricular mucosa of ducks at acidic pH. The presence of enzyme maltase in the mucosal extracts of oesophagus and crop of ducks were also noted.

Pisharody (1970) quantified the activity of amylase of small intestinal mucosa of both chicks and ducklings and found that ducklings exhibited higher enzyme activity. The optimum temperature for intestinal amylase activity for both the species was 41°C and the optimum pH for chicken and duckling intestinal

amylase was 7.2 and 7.0 respectively. High protein diet did not influence the intestinal amylase activity in both chicks and ducklings, but fat diet decreased amylase activity in ducklings, whereas in chicks the activity was unchanged. The chicken and duckling intestinal amylase was 48.75 and 70.27 Somogyi units/ g of tissue respectively.

Pisharody (1970) quantified the presence of protease in the mucosal extracts of proventriculus of both chicks and ducklings. The optimum pH for pepsin activity of chicks and ducklings were 2 and 2.4 respectively and optimum temperature was 41^0C. The protease activity in chicken and duckling was 403.63 and 325.17 PU/g of tissue respectively. No appreciable increase in protease activity of pepsin was observed both in chicks and ducklings maintained on a high protein diet compared to that of birds on a normal diet.

Hulan and Bird (1972) reported an increase in the activity of lipase in the pancreatic juice of birds by the intake of a high fat diet.

Goel and Jain (1993) studied the pancreatic lipase activity between avian and mammalian specious. The pancreatic lipase activities were 120 and 60 units/ g fresh tissue respectively.

Sharatchandra *et al.* (1995) reported that a single oral dose of mint leaf and garlic oil to rats significantly stimulated lipase activity by 4-6 fold and 2.5-5 fold, respectively compared to control.

Beena (2000) studied the lipase activity of pancreas of male and female Japanese quail. The mean lipase activities of pancreas of adult male and female quails were 73.37 and 38.40 LU/g of tissue respectively. The males had a significantly higher lipase activity than females.

Beena and Philomina (2000a) studied the protease activity in proventriculus of Japanese quail. The mean protease activities of proventricular

mucous membrane of adult male and female quails were 185.67 PU/g of tissue and 223.31 PU/g of tissue respectively.

Beena and Philomina (2000b) studied the lipase activity in pancreas and small intestine of six week old Japanese quail. Pancreatic lipase activities in male and female Japanese quail were 73.37 LU/g of tissue and 38.40 LU/g of tissue respectively. Lipase activities of intestinal mucous membrane of adult male and female quail were 38 LU/g of tissue and 12.44LU/ g of tissue respectively.

Platel and Srinivasan (2000) reported that dietary curcumin (0.5 per cent), capsaicin (1.5 mg per cent), piperine (20 mg per cent), ginger (50 mg per cent), cumin (1.25 per cent) and asafoetida (250 mg per cent) enhanced pancreatic lipase activity in rats compared to control.

Platel and Srinivasan (2001) stated that pancreatic lipase was stimulated in rats by ajowan up to 26 per cent; on the contrary, the lipase activity was lowered by dietary mint and garlic to an extent of about 42 per cent.

Platel *et al.* (2002) reported that pancreatic lipase activity was increased by 40 per cent when all the spice mixes (Spice mix I – turmeric, coriander, red chilly, black pepper and cumin; Spice mix II - turmeric, coriander, red chilly, black pepper, cumin and ginger; Spice mix III - turmeric, coriander, red chilly, black pepper, cumin and onion) were given through the diet to rats.

Platel and Srinivasan (2004) reported that rat pancreatic amylase activity was elevated up to 96 per cent by curcumin compared to control group.

Namagirilakshmi (2005) conducted a study on effect of turmeric powder on intestinal amylase activity and lipase activity of broilers. The birds supplemented with turmeric powder at the rate of 0.25, 0.5, 0.75 and 1.25 per cent incorporated in the feed to T2, T3, T4 and T5 respectively, while T1 was control. Highest amylase activity was observed in experimental group than control group. The highest enzyme activity was in 0.5 per cent supplemented group (92.33 U/ml)

and control group had the lowest enzyme activity (69.32 U/ml). No significant difference was noted between the two groups. The lipase activity in control group was 5.77 U/ml and lowest enzyme activity was 3.22 i.e. the birds supplemented with 0.25 per cent turmeric powder in feed. There was significant difference noted between the two groups in lipase activity.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL BIRDS

Sixty, day - old broiler chicks (Vencob strain) procured from Mannuthy Hatcheries, Nettissery reared under standard managemental condititions in battery brooder formed subjects of this study. They were divided into six groups viz; G1, G2, G3, G4, G5 and G6 each comprising of 10 birds. They were fed with commercial broiler starter ration for the first four weeks and then with finisher ration for two weeks (Table 1.). The chemical composition of the above rations is presented in Table 2. The study was conducted upto sixth week of age.

3.2 EXPERIMENTAL RATION

Commercially available root of *Withania somnifera* (Aswagandha) was dried under shade, powdered and used for the study (Plate1 and Plate 2). Proximate analysis of *W. somnifera* was also carried out as per AOAC (1990) (Table 3.). *W. somnifera* was incorporated at the rate of 5 g and 10 g / kg of feed in starter and finisher broiler ration.

3.3 EXPERIMENTAL DESIGN

W. somnifera root powder was incorporated in starter ration as well as in finisher ration of birds in all the groups except G1 and G4. Stress was induced in G4, G5 and G6 from fifth week of age by reducing the floor space of each bird by 1/3rd of the optimum requirement (1116 cm^2 / bird). The groups and treatments are given in Table 4.

All birds were immunized using 1ml of 7 per cent sheep red blood cell (SRBC) intravenously on 23 rd day (5 day before subjecting to stress) for the evaluation of immune status. The studies on liver and intestinal enzymes were conducted after slaughter.

Plate 1. ***Withania somnifera*** **plant**

Plate 2. ***Withania somnifera*** **roots**

Table 1. Composition of broiler chicken ration

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

* Keyes mineral mixture (R) M/s Kerala Solvent Extractions Limited, Irinjalakuda, each gram contains
Calcium 32 per cent, Phosphorus 6 per cent, Magnesium 1000 ppm, Cobalt 60 ppm, Zinc 2600 ppm, Iron 0.1 per cent, Iodine 100 ppm, Copper 100 ppm and Manganese 2700 ppm

** Indomix (R) M/s. Nichola Piramal India Pvt. Ltd, Mumbai India each gram contains Vit A- 82500 IU, Vit B_2- 50 mg, Vit D_3 -12,000 IU and Vit K – 10 mg.

Table 2. Proximate principles of broiler chicken ration (On dry matter basis)

Sl. No.	Nutrients	Standard broiler ration (per cent)	
		Starter	Finisher
Analysed values*			
1.	Moisture	6.60	7.60
2.	Crude protein	23.40	19.65
3.	Ether extract	7.65	9.43
4.	Crude fibre	2.86	4.14
5.	Nitrogen free extract	54.79	53.34
6.	Total ash	11.30	13.44
7.	Acid insoluble ash	3.75	3.65
8.	Calcium	1.58	1.41
9.	Phosphorus	0.86	0.77
Calculated values			
10.	Metabolizable energy (kcal/kg)	2899.00	2981.00
11.	Lysine (per cent)	1.50	1.00
12.	Methionine (per cent)	0.53	0.40
13.	Manganese (mg/kg)	104.00	102.00

- Mean of eight samples

Table 3. Proximate analysis of *Withania somnifera root*

Parameters	Per cent
Moisture	4.78
Dry matter	95.21
Total ash	5.58
Acid insoluble ash	0.41
Crude protein	14.10
Crude fat	10.93
Crude fibre	0.67

Table 4. Experimental design (n=10)

Groups	Treatments
G1	Normal birds fed with standard broiler ration (BIS specification).
G2	Normal birds fed with standard broiler ration supplemented with *W. somnifera* at the rate of 5 g / kg of feed.
G3	Normal birds fed with standard broiler ration supplemented with *W. somnifera* at the rate of 10 g / kg of feed.
G4	Stressed birds fed with standard broiler ration (BIS specification).
G5	Stressed birds fed with standard broiler ration supplemented with *W. somnifera* at the rate of 5 g / kg of feed.
G6	Stressed birds fed with standard broiler ration supplemented with *W. somnifera* at the rate of 10 g / kg of feed.

3.4 BLOOD COLLECTION

Blood samples (5ml) were collected by wing vein puncture of birds with and without anticoagulant (EDTA) from all birds. Blood samples were collected five days before immunization and one day (29th day) before the induction of stress as well as on day 1, 3, 6, 9, 12 and 15 after the induction of stress. The separated serum was stored at -4^0C till analysis.

3.5 BODY WEIGHT AND FEED CONSUMPTION

The body weight of the birds was recorded at weekly intervals from first week to sixth week of age. The feed supplied and left over in each week was recorded and the feed consumption of the birds was calculated.

3.6 FEED CONVERSION EFFICIENCY

Feed conversion efficiency was calculated based on the data of body weight gain and feed intake.

$$\text{Feed conversion efficiency} = \frac{\text{Feed consumption (g)}}{\text{Body weight gain (g)}}$$

3.7 SLAUGHTER STUDIES

Slaughter studies were conducted at seventh week of age. After final blood collection birds were sacrificed by cervical dislocation. Bursa, spleen, adrenal gland, giblet (heart, liver and gizzard) were excised out and were thoroughly washed, mopped and weighed. A piece of liver from each bird was excised and stored at -4^0C for estimation of tissue lipid peroxidation and reduced glutathione levels. The proventriculus and duodenum were exposed, washed with ice-cold normal saline; mucous membrane was carefully scrapped out from proventriculas and duodenal regions and were used for protease and amylase estimation. The whole pancreas was homogenized using normal saline for lipase estimation.

3.8 ESTIMATION OF HAEMATOLOGICAL PARAMETERS

3.8.1 Haemoglobin

Haemoglobin level was determined by standard procedure of cynmethaemoglobin method (Dacie and Lewis, 1968).

3.8.2 Volume of Packed Red Cells (VPRC)

Volume of packed red blood cells was estimated by micro haematocrit method (Feldman *et al.*, 2000).

3.8.3 Total Erythrocyte Count and Leucocyte Count

The method described by Natt and Herrick (1952) was followed for total erythrocyte count and total leukocyte count.

Composition of Natt and Herrick's fluid

Sodium chloride	3.88 g
Potassium chloride	2.50 g
Di sodium hydrogen phosphate	1.44 g
Potassium dihydrogen phosphate	0.25 g
Formalin (37 per cent)	7.50 ml
Methyl violet 2B	0.10 g
Distilled water	1000 ml

The above preparation was stirred over night, filtered and used for the study.

3.8.3.1 Enumeration of RBC

Blood was diluted with Natt and Herrick's reagent in the ratio of 1:200 using RBC diluting pipette. After mixing, the diluted blood was loaded on a haemocytometer and kept for 5 minutes. RBC's located in 4 corner and central small squares of medium sized squares were counted (A).

Total RBC = A X 10^6/ µl of blood

3.8.3.2 Enumeration of WBC

The same procedure quoted in 3.8.3.1 was followed. After keeping for 5 minutes white blood cell located in the nine large squares in the ruled area were counted (B)

Total WBC = (B+10 per cent B) X 200/ µl of blood

3.8.4 Mean Corpuscular Volume (MCV)

Mean corpuscular volume is the average or mean volume of red cell. It is calculated from the following formulae (Swenson and Reece, 1996). MCV is expressed in fl.

$$\frac{\text{Volume of packed red cell in per cent}}{\text{RBC x } 10^6 / \mu l} \text{ x } 10$$

3.8.5 Mean Corpuscular Haemoglobin (MCH)

Mean corpuscular haemoglobin determine the average haemoglobin content in a single red cell. It is calculated by the following formulae (Swenson and Reece, 1996). MCH is expressed in pg.

$$\frac{\text{Haemoglobin in gram per cent}}{\text{RBC x } 10^6 / \mu l} \text{ x } 10$$

3.8.6 Mean Corpuscular Haemoglobin Concentration (MCHC)

It represents relationship between red cell volume and its degree or percentage saturation with heamoglobin. It is calculated by the following formulae (Swenson and Reece, 1996). MCHC is expressed in per cent.

$$\frac{\text{Haemoglobin in gram per cent}}{\text{Volume of packed red cell in per cent}} \text{ x } 100$$

3.8.7 Heterophil: Lymphocyte Ratio

Air dried blood smear was stained with Leishman- Giemsa stain solution and different leucocytes were counted and their percentages were determined. Heterophil: Lymphocyte ratio was calculated (Gross and Siegel, 1983).

3.8.7.1 Preparation of Leishman- Giemsa Stain

Ground 0.15 g Leishman stain powder and 30 mg of Giemsa powder with small amount of acetone free methyl alcohol until an even suspension of stain was obtained. A total of 100 ml of acetone free methyl alcohol was added to produce a complete solution. The stain solution was filtered and stored in dark bottle and aged for 3 weeks prior to use.

3.8.7.2 Method of Staining

The stain solution was filtered and the air dried blood film was flooded with undiluted stock solution of Leishman- Giemsa stain and kept for 45 seconds to fix. The stain was diluted to double the volume with buffered distilled water, mixed by gentle blowing and was allowed to stain for 3 minutes. The slide was washed in distilled water, back of slide was wiped to remove excess stain and the slide was air dried in upright position.

3.9 ESTIMATION OF BIOCHEMICAL PARAMETERS

3.9.1 Serum Protein Profile

3.9.1.1 Serum Total Protein

Serum total protein was estimated by Biuret method (Henry *et al.*, 1957) using kits of Agappe diagnostics Pvt. Ltd, Maharashtra.

3.9.1.2 Serum Albumin

Serum albumin was estimated by the method of Doumas *et al.* (1971) using kits of Agappe Diagnostics Pvt. Ltd, Maharashtra.

3.9.1.3 Serum Globulin

Serum globulin content was calculated as the difference between serum total protein and albumin content.

3.9.1.4 Albumin: Globulin Ratio

The albumin /globulin ratio was calculated using the following formulae

$$\frac{\text{Concentration of Albumin (g/dl)}}{\text{Concentration of Globulin (g/dl)}}$$

3.9.2 Serum Enzymes

3.9.2.1 Serum Alanine Amino Transferase (ALT)

The level of ALT in the serum was determined by U V Kinetic method (Bergmayer, 1974) utilizing the kit supplied by Agappe diagnostics Pvt. Ltd, Maharashtra.

3.9.3 Serum Lipid Profile

3.9.3.1 Serum Total Cholesterol

The concentration of serum total cholesterol was estimated by cholesterol phenol aminoantipyrine (CHOD – PAP) method as suggested by Richmond (1973) using kits of Agappe Diagnostics Pvt. Ltd, Maharashtra.

3.9.4 Serum Glucose

Serum glucose was estimated by Glucose oxidase - peroxidase (GOD-POD) method as determined by Mayne (1994) using kits of Agappe Diagnostics Pvt. Ltd, Maharashtra.

3.9.5 Serum C - reactive protein (CRP) level

The Avitex – C reactive protein (CRP) latex test kit (Qualigens Diagnostics, India) based on the method suggested by Wadsworth *et al.* (1984)

was used for the semi quantification of the CRP in the serum of experimental birds. C-reactive protein was done only in fifth week and serum was taken from only normal birds, stressed birds and stressed birds supplemented with *W. somnifera.* Serum samples of birds from each group were checked for the presence of CRP. Using isotonic saline (50 μl), serial dilution of serum (50 μl) was prepared (1/2, 1/4, 1/8 and 1/16) on avitex latex test card. Transferred 50 μl of each serum dilution to the test circle of avitex latex test card. Latex reagent (50 μl) was added to the test circle and mixed. The test card was gently and evenly rocked and rotated for 2 minutes and examined for agglutinated particles macroscopically and then confirmed microscopically. The CRP concentration was then calculated by multiplying the dilution factor (i.e., 2, 4, 8 or 16) with the detection limit value. i.e., 6 to get the mg/dl concentration of CRP. This was calculated only on ninth day of stress.

3.9.2 Tissue

3.9.2.1 Estimation of Lipid Peroxides in Liver Tissue

Liver lipid peroxidation (LPO) levels were assessed by determining small amounts of malondialdehyde (MDA) produced during peroxidation, and the concentration in liver was expressed as nanomole of MDA formed per milligram protein (Okhawa *et al.*, 1979).

Principle

Thiobarbituric acid (TBA) reacts with lipid peroxides and malondialdehyde to form a red coloured pigment that could be determined by colorimetry. Tetramethoxy propane (TMP) was used as the standard, since it could be converted to malondialdehyde quantitatively by reacting with TBA. After heating at 95°C for 60 min, the red pigment produced was extracted with n-butanol-pyridine mixture and estimated by the absorbance at 532nm.

Chemicals required

TBA and TMP (Tetramethoxy propane) were purchased from Himedia Laboratories Pvt. Ltd., Mumbai. Sodium dodecyl sulphate (SDS) was procured from Sigma – Aldrich, Bangalore; and all the other chemicals were purchased from Merck India Ltd.

Reagents

1. 8. 1 per cent SDS – 8.1 g of SDS was dissolved in 100 ml distilled water.
2. 20 per cent acetic acid solution, pH adjusted to 3.5 with NaOH.
3. 0.8 per cent aqueous solution of TBA- 0.8 g of TBA was dissolved in 100 ml distilled water.
4. 1.15 per cent KCl- 1.15 g of KCl was dissolved in 100 ml distilled water.

Procedure

Preparation of tissue homogenate

Homogenates of liver was prepared in a ratio of 1g of wet tissue to 9 ml of 1.15 per cent KCl solution using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was used for the estimation of lipid peroxides.

To the 100 μl of the supernatant, added 200 μl of 8.1 per cent SDS, 1.5 ml of 20 per cent acetic acid solution (pH, 3.5) and 1.5 ml of 0.8 per cent aqueous solution of TBA. The mixture was made up to 4 ml with distilled water, and heated in a water bath at 95°C for 60 minutes. After cooling under tap water, 1 ml of distilled water and 5 ml of n-butanol were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, optical density of the organic layer was taken at 532 nm.

Preparation of standard curve

Standard curve was prepared using concentrations varying from 0.30 nM to 9.74 nM of TMP in deionised double distilled water. A graph was plotted with optical density and concentration of the standards. The level of lipid peroxides was read directly from the standard curve and expressed as nmol of malondialdehyde /g wet tissue.

3.9.2.2 Estimation of Reduced Glutathione in Liver Tissue

Level of reduced glutathione in tissue homogenate was estimated by the method of Moron *et al.* (1979).

Principle

Reduced gluathione is measured by its reaction with 5-5' dithiobis 2-nitrobenzoic acid (DTNB) by thiol groups giving 2 nitro 5 mercaptobenzoic acid having an intense yellow coloured complex, which absorbs maximally at 412 nm.

Chemicals required

Disodium hydrogen phosphate and DTNB were purchased from Himedia Laboratories Pvt. Ltd., Mumbai. Trichloro acetic acid (TCA) was procured from Qualigens Fine Chemicals, Glaxo Smith Kline Pharmaceuticals Ltd, Mumbai. Mono and disodium hydrogen phosphates were used for the preparation of Phosphate buffer.

Reagents

1. Phosphate buffer (pH 8, 0.2 M)

Solution A: Dissolved 3.12 g of NaH2PO4 $2H_2O$ in 100 ml distilled water.

Solution B: Dissolved 28.39 g of Na_2HPO_4 in 100 ml distilled water.

Mixed 5.3 ml of solution A and 94.7 ml of solution B.

2. DTNB (0.6mM) - Freshly dissolved 12 mg DTNB in 50 ml of the buffer.

3. Twenty five per cent and five per cent Tri chloro acetic acid (TCA): Dissolved 25 g TCA in 100 ml distilled water and 5 g TCA in 100 ml distilled water respectively.

4. Reduced glutathione (GSH) standard – 50 mg / dl

Procedure

Preparation of tissue homogenate

Homogenated liver tissues were prepared in a ratio of 1 g of wet tissue to 9 ml phosphate buffer (pH, 8) using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was used for the estimation of reduced glutathione.

Added 125 µl of 25 per cent TCA to 500 µl of supernatant for the precipitation of proteins. The tubes were cooled on ice for 5 minutes and the mixture was further diluted with 575 µl of 5 per cent TCA. Centrifuged the tubes for 5 minutes and 300 µl of resulting supernatant was taken for the estimation of reduced glutathione. The volume of aliquot was made up to 1 ml with 0.2 M phosphate buffer (pH, 8). Added 2 ml of freshly prepared 0.6 mmol DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. The level of reduced glutathione was determined from the standard curve.

Preparation of standard curve

Standard curve was prepared using concentrations varying from 10 µg to 50 µg of reduced glutathione which was dissolved in five per cent TCA. The volume of standard solution was made upto1ml with 0.2 M Phosphate buffer (pH, 8). Added 2 ml of freshly prepared 0.6 mmol DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. A graph was plotted between absorbance and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of of GSH was read directly from the calibration curve and expressed as µg/g wet tissue of liver.

3.10 IMMUNOLOGICAL PARAMETERS

Reagent used for immunological studies

Phosphate buffer saline (PBS)

Sodium chloride	- 8.00 g
Potassium chloride	- 0.20g
Disodium hydrogen phosphate	- 1.44 g
Potassium hydrogen phosphate	- 0.02 g
Distilled water	- 1000ml

The pH was adjusted to 7.4 with 1.0 N HCl or NaOH

PBS – EDTA solution

EDTA	- 20 mg
PBS	- 100 ml

Sterilized by autoclave.

Trypsin solution

Trypsin	- 200 mg
Glucose	- 20 mg
PBS – EDTA	- 100 ml

Sterilized by filtration

Alsever's solution

Dextrose	- 2.05 g
Sodium citrate	- 0.80 g
Sodium chloride	- 0.42 g
Distilled water	- 100ml

The pH was adjusted to 6.1 with 10 per cent citric acid, autoclaved at 10 lbs for 15 min and stored at 4°C.

3.10.1 Estimation of Circulating Antibody Titre

3.10.1.1 Preparation of Antigen

The antigen used was SRBC collected from adult healthy sheep (Madras Red breed). Whole blood (5 ml) was drawn from the donor by jugular vein puncture, diluted in an equal volume of Alsever's anticoagulant and centrifuged at low speed (1000 rpm) to sediment the erythrocytes. The overlying plasma and suspended leucocytes were aspirated out and discarded. The pelleted blood was resuspended in physiological saline (0.90 per cent) and centrifuged at 3000 rpm for 10 min. The washing was repeated four times, and finally the pellets were resuspended in physiological saline and stored as stock suspension under refrigeration.

3.10.1.2 Immunization of Birds

All birds (Group I to VI) were immunized with 1 ml of 7 per cent SRBC i.v, (prepared from the stock solution), five days ahead to stress. A volume of 2 ml blood from wing vein was collected from each bird on alternate days from day 5 and 1 during prestress period and day 1, 3, 6, 9, 12 and 15 days after stress. Serum separated from each group was utilized for estimating circulating antibody titres.

3.10.1.3 Hemagglutinin (HA) Assay

A microtitre plate procedure was employed for finding antibody titres (Mehra and Vaidhya, 1993). The heat inactivated (56°C for 30 min) serum sample (100μl) was serially two-fold diluted with PBS in U- shaped bottom 96 well microtire plate (Tarson's India Ltd.). An equal volume of 2 per cent trypsinized erythrocyte suspension was then added to each well and incubated for 1 hour at room temperature. The degree of agglutination was evaluated macroscopically and the HA titres were expressed as the $\log_2$ of the reciprocal of the highest dilutions giving visible agglutination.

3. 11 GASTROINTESTINAL PARAMETERS

3.11.1 Preparation of Tissue Homogenate

At seventh week of age, the birds were sacrificed by cervical dislocation, the body cavity was exposed and the whole of the gastro intestinal tract was dissected out. The pancreas was taken and weighed immediately. The different regions of the digestive tract (proventriculus and duodenum) were exposed washed out with ice cold normal saline and then mopped off the moisture from the inner side. The narrow membrane was carefully scrapped out from these different regions and weight of these tissue scrapings were taken immediately. Weighed the whole of the pancreas and it was homogenized with ice cold normal saline and the final volume of the homogenate of each sample was made upto 25 ml.

3.11.2 Estimation of Proteases

Principle

The tissue homogenate is incubated with casein solution. The unaltered casein is salted out. The filtrate from digestion products of casein is estimated titrimetrically (Volhard and Lohlein-Hawk *et al.*, 1954).

Reagents

1. Casein solution 5 per cent

To 1000 ml of volumetric flask, added 50 g of the casein powder and 500 ml of glass distilled water to it, mixed well and allowed to stand for 3 hours. Added 40 ml of 1 N sodium hydroxide solution and the volume was made up to 1 litre, warmed gently until it became clear and then heated rapidly to 85 to 90^{0} C to destroy any proteinases. The solution was then transferred to a stopperd bottle, added a few drops of toluene as preservative and stored in a refrigerator.

2. Hydrochloric acid 1 N

This was used for coagulation of the protein. Nine ml of concentrated hydrochloric acid was diluted to 100 ml and standardized against 1 N NaOH using phenolphthalein as indicator.

3. Sodium sulphate solution 20 per cent

Two hundred g of anhydrous sodium sulphate was dissolved in glass distilled water in a 100 ml volumetric flask and the volume was made up to the mark. This was used for precipitation of casein.

4. Sodium hydroxide solution 0.1 N

Saturated solution of sodium hydroxide (5.9 ml) was diluted to 1000 ml with glass distilled water and standardized against 0.1 N oxalic acid using phenolphthalein as indicator. Appearance of pale pink colour is the end point of titration.

5. Phenolphthalein Indicator

Phenolphthalein (0.5g) was dissolved in 50 ml of absolute alcohol and final volume was made up to 100 ml with glass distilled water.

Procedure

Into each one of two big test tubes introduced 1.1 ml of 1 N hydrochloric acid and diluted with glass distilled water to 15 ml. With constant shaking added 10 ml of caesin solution to each tube. Five ml of the tissue homogenate was added to one of the tube labeled as "test". The other tube formed the "control". The tubes were kept in water both at 40°C for one hour. At the end of this period, they were taken out and 5 ml of the homogenate was added to the control. Added 10 ml of 20 per cent sodium sulphate solution to each tube and filtered through whatman No.1 filter paper, to a clean dry tube. Titrated 10 ml of the filtrate in each tube with 0.1 N sodium hydroxide solution using phenolphthalein as

indicator. Appearance of pale pink colour was the end point of titration. Protase activity was expressed in "pepsin" unit/ g of tissue.

Calculation

Protease activity (pepsin units/g of tissue) = $\frac{[(T - C)\,4]^2 \times 5}{W}$

Where,

T = Titre value of test (ml)

C = Titre value of control (ml)

W = Weight of tissue (g)

3. 11.3 Estimation of Amylase

Principle

An aliquot of the tissue homogenate is incubated at 37° C with 0.4 mg of starch and the loss in blue colour which the starch gives with iodine solution is taken as a measure of the extend to which starch has been digested by the amylase.

The amylase activity is expressed in terms of "units" (somogyi units) of amylase. The "unit" is defined as the amount of amylase which would destroy mg of starch in 15 minutes (King and Wootton., 1959).

Reagents

1. Starch solution (0.1 per cent)

Hundred mg of pure analar soluble starch was mixed with 5 ml of glass distilled water in a 100 ml volumetric flask and three lots of 30 ml boiling glass distilled water were added, mixed well cooled, diluted to the mark and preserved in the freezing chamber of a refrigerator.

2. 0.02 M Phosphate buffer pH 7.0

Anhydrous disodium hydrogen phosphate (1.736 g) and 1.059 g of monopotassium dihydrogen phosphate were dissolved in 1000 ml of glass distilled water and pH was adjusted to 7.0. Two ml of chloroform was added to the solution as a preservative and stored in a refrigerator.

3. Buffered substrate

Five volume of phosphate solution was mixed with 4 volume of starch solution prepared fresh.

4. Stock iodine solution 0.1 N

Pure sublimated iodine (13.5 g) was dissolved in a solution of 24 g of potassium iodine in 100 ml of glass distilled water contained in a litre volumetric flask. The solution was diluted to the mark and standardized against 0.1 N sodium thiosulphate using starch as an indicator. Disappearance of deep purple colour of iodine solution was the end point of the titration.

5. Dilute iodine Solution

Fifty g of potassium fluoride was dissolved in glass distilled water in a liter volumetric flask and 100 ml of the stock iodine solution was added to it. The volume was made up to the mark and the solution was stored in a brown bottle in a refrigerator.

Procedure

Buffered substrate (0.9 ml) was warmed in a glass stoppered tube and was labelled "Test", kept in a water bath at 37°C for 2 minutes. Tissue homogenate (0.1 ml) was then mixed with it and incubated at 37° C for 15 minutes. The tube was then taken out of the water bath, cooled, added 8.6 ml of glass distilled water followed by 0.4 ml of dilute iodine solution and mixed well.

Blank was prepared in a similar manner as the "Test", except 0.1 ml of the tissue homogenate was added to the substrate after incubation and addition of dilute iodine solution.

The "Test" and "Blank" were read in a spectrophotometer at 680 nm within five minutes after addition of iodine solution after setting the instrument to zero with distilled water.

Calculation

$$\text{Amylase activity (Somogyi units / g of tissue)} = \frac{B - T}{B} \times \frac{0.4 \times 25}{5 \times 0.1 \times W}$$

Where,

B = Reading of blank (nm)

T = Reading of the test (nm)

W = Weight of the tissue (g)

3.11.4 Estimation of Lipase

Principle

The tissue homogenate is incubated with olive oil emulsion substrate. The lipase activity results in splitting off the glyceryl-fatty acid ester bond with the liberation of free fatty acids. The amount split off is determined by titrating the liberated fatty acids with standard alkali using phenolphthalein as the indicator (Boutwell, Jr. 1962).

Reagents

1. Olive oil emulsion

Dissolved 12.50 g of gum acacia and 0.20 g of sodium benzoate in glass distilled water. Added 50 ml of pure olive oil to it. Emulsified the mixture in a homogenizer until a smooth emulsion was obtained and stored in a refrigerator and shaken well before use.

2. Phosphate buffer 0.067 M (pH, 7.0)

Dissolved 5.51 g of anhydrous disodium monohydrogen phosphate and 3.83 g of anhydrous monopotasium dihydrogen phosphate in glass distilled water in a liter volumetric flask and the volume was made upto the mark. Adjusted the pH of the solution to 7.0 using a pH meter. One ml of toluene was added as a preservative and stored in a refrigerator.

3. Sodium hydroxide solution 0.05 N

Sodium hydroxide 0.1 N was standardized against 0.1 N oxalic acid using phenolphthalein as indicator. Appearance of pale pink colour is the end point of titration. Diluted 500 ml of 0.1N NaOH to a litre with glass distilled water, in a volumetric flask.

Procedure

The substrate buffer mixture was prepared by stirring 5 volumes of phosphate buffer with 1 volume of olive oil emulsion. Transferred 12 ml portions of the substrate buffer mixture into 2 tubes warmed the tubes in a water bath to 37°C. Added 5 ml of the tissue homogenate to one of the tubes and mixed thoroughly by gentle inversion. Second tube served as the blank. Incubated both the tubes at 37°C for 24 hours. At the end of incubation the tubes were taken and 5 ml of the tissue homogenate was added to the blank. Titrated the mixture with 0.05 N sodium hydroxide using six drops of phenolphthalein indicator. Appearance of pale pink colour was the end point of titration.

Calculation

A lipase unit is defined as that quantity of enzyme which releases acid equivalent to 1 ml of N/20 NaOH in 24 hours from an olive oil substrate.

$$\text{Lipase activity (Lipase units / g of tissue)} = \frac{(T-B)\,5}{W}$$

Where,

T = Titre value of test (ml)

B = Titre value of blank (ml)

W = Weight of tissue (g)

3.12 STATISTICAL ANALYSIS

The data were analyzed by using the statistical techniques, Analysis of Variance and Paired t-Test (Snedecor and Cochran, 1994).

Results

4 RESULTS

4.1 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON BODY WEIGHT OF BROILER CHICKEN

4.1.1 Body Weight

The weekly body weights (mean ± SE) of all the experimental groups from day old to six weeks of age are presented in Table 5 and Fig1. The G1 (normal) (107.00 ±7.79 g) chicken had a significant ($P<0.05$) increase in body weight compared to G3 chicken (normal + 10 g *W. somnifera*/ kg feed) (77.00±5.17 g) and no significant difference with G2 (normal + 5 g *W. somnifera*/ kg feed) (113.50±10.22 g) chicken and G4 chicken (stressed) (101.50±5.06 g). G2 chicken had a significant ($P<0.05$) increase in body weight than G3 chicken. G4 chicken had no significant difference with G5 chicken (stressed +5 g *W. somnifera*/ kg feed) (88.80±5.44 g) and G6 chicken (stressed + 10 g *W. somnifera*/ kg feed). G5 chicken had no significant difference with G6 chicken (100.00±.00 g).

There was no significant difference in body weight between groups at second, third, fourth, fifth and sixth week of age. The values within the groups were compared with the previous week body weight. G1 to G6 chicken showed a significant ($P<0.05$) increase in body weight between first, second, third, fourth, fifth and sixth week of age but G4 chicken had no significant difference at sixth week of age. The mean value of G1, G2, G3, G4, G5 and G6 was 1505.00± 97.47 g, 1490.00± 51.29 g, 1484.00± 92.4 g, 1333.00± 38.36 g, 1394.50± 41.06 g, 1340.50±104.68 g respectively at sixth week of age.

4.1.2 Weight gain

The weight gains (mean ± SE) of all the experimental groups from day old to six weeks of age are presented in Table 6 and Fig 2. At first week of age the G1

(50.00±1.10 g) chicken had no significant difference with G2 (65.20±8.94 g) and G4 chicken (53.20±4.39 g) but had a significant ($P<0.05$) increase than G3 (31.60±5.72 g) chicken. G2 chicken had a significant ($P<0.05$) increase than G3 chicken. G4 chicken had no significant difference with G5 and G6 chicken. G5 chicken expressed no significant difference with G6 chicken.

There was a no significant difference in weight gain between groups at second, third, fourth and fifth and sixth week of age. The weekly weight gain within groups was compared with their previous week. G1 chicken had a significant ($P<0.05$) gain in body weight increase in second (135.50±16.82 g) and fourth (324.50±22.87 g) week of age but not in third, fifth, and sixth week of age. G2 chicken had a significant ($P<0.05$) increase in second (179.00±18.91 g) and fourth (358.00±24.77g) week of age but not in third, fifth and sixth week of age. G3 chicken had a significant ($P<0.05$) increase in second (153.00±13.69 g), fourth (360.00±21.92 g), fifth (278.00±30.30 g) and sixth (449.00±54.17 g) week of age but not in third week of age. G4 chicken had a significant ($P<0.05$) increase in second (143.50±9.07 g), third (215.50±23.86 g), fourth (346.00±19.97 g) and sixth (180.00±44.10 g) week of age and no significant difference in fifth week of age. G5 chicken had a significant ($P<0.05$) increase in second (176.20±23.49 g) and fourth week (353.00±22.15 g) of age and not in third, fifth and sixth week of age. G6 chicken had a significant ($P<0.05$) increase in second (137.00±13.85 g), third (205.50±19.80 g), fourth week (325.00±14.94 g) and sixth week (322.00±36.33 g) of age and no significant difference in fifth week of age.

Table 5. Weekly body weight (g) of experimental groups (Mean ± SE, n=10)

Weeks / Groups	Body weight (g)						
	0	1	2	3	4	5	6
G 1	49.50±1.42	107.00^{bc*} ±7.79	252.50*±18.43	472.00*±31.85	837.00*±45.97	1162.00*±44.59	1505.00*±97.47
G 2	48.30±1.65	113.50^{c*} ±10.22	292.50*±14.46	512.50*±15.48	850.50*±28.81	1117.00*±39.36	1490.00*±51.29
G 3	46.20±1.35	77.00$^{a\,*}$±5.17	226.00*±16.19	432.50*±29.83	757.00*±48.01	1055.00*±64.50	1484.00*±92.49
G 4	48.30±1.22	101.50$^{bc\,*}$±5.06	245.00*±5.00	460.50*±25.28	806.50*±33.50	1173.00*±32.04	1333.00±38.36
G 5	50.50±1.75	88.80$^{ab\,*}$±5.44	265.00*±25.06	462.50*±26.68	817.50*±31.97	1150.00*±40.82	1394.50*±41.06
G 6	49.60±0.96	100.00$^{bc\,*}$±.00	237.00*±13.85	442.50*±25.83	767.50*±33.44	1018.50*±71.17	1340.50*±104.68

G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed +5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.
Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.
In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.
* denotes significant difference (P<0.05) within a row from previous week.

Table 6. Weekly weight gain of experimental groups (Mean ± SE, n=10)

	Weight gain (g)					
Weeks / Groups	1	2	3	4	5	6
G 1	$50.00^{bc}\pm1.10$	$135.50^{*}\pm16.82$	207.00 ± 25.48	$324.50^{*}\pm22.87$	330.00 ± 30.97	$323.00^{abc}\pm76.84$
G 2	$65.20^{c}\pm8.94$	$179.00^{*}\pm18.91$	240.00 ± 28.19	$358.00^{*}\pm24.77$	266.50 ± 41.35	$373.00^{bc}\pm58.58$
G 3	$31.60^{a}\pm5.72$	$153.00^{*}\pm13.69$	206.50 ± 23.52	$360.00^{*}\pm21.92$	$278.00^{*}\pm30.30$	$449.00^{c*}\pm54.17$
G 4	$53.20^{bc}\pm4.39$	$143.50^{*}\pm9.07$	$215.50^{*}\pm23.86$	$346.00^{*}\pm19.97$	366.50 ± 37.12	$180.00^{a*}\pm44.10$
G 5	$38.50^{ab}\pm4.05$	$176.20^{*}\pm23.49$	207.50 ± 27.65	$353.00^{*}\pm22.15$	334.50 ± 42.32	$244.50^{ab}\pm23.03$
G 6	$50.40^{bc}\pm0.96$	$137.00^{*}\pm13.85$	$205.50^{*}\pm19.80$	$325.00^{*}\pm14.94$	251.00 ± 46.78	$322.00^{abc*}\pm36.33$

G1- Normal , G2- Normal +5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed with 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly ($P>0.05$) between groups.

In a column if there is no superscript it means no significant difference ($P>0.05$) among six treatments.

* denotes significant difference ($P<0.05$) within a row from previous week

Figure 1. Weekly body weight of experimental groups (Mean ± SE, n=10)

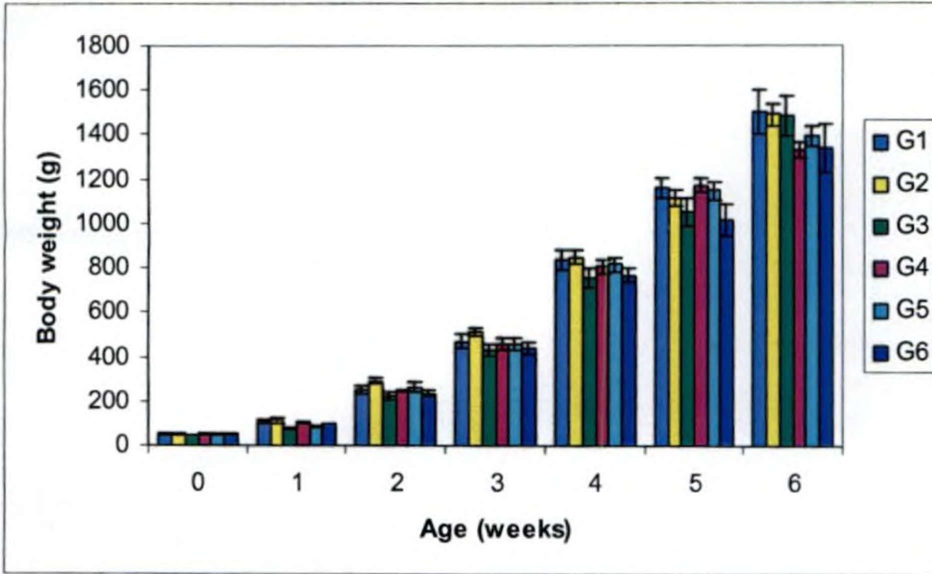
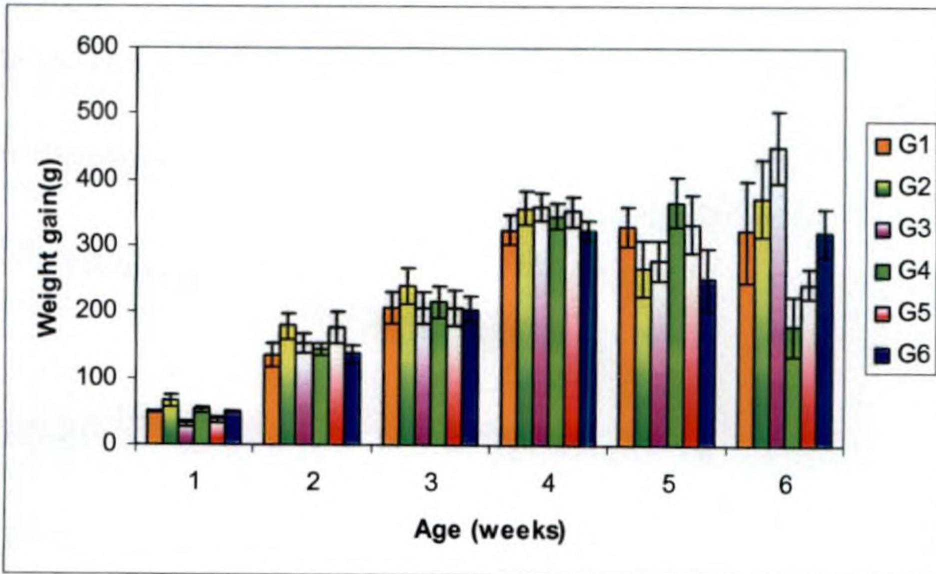


Figure 2. Weekly weight gain of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

4.2 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON FEED CONSUMPTION AND FEED EFFICIENCY OF BROILER CHICKEN

4.2.1 Feed Consumption

The feed consumption of all the experimental groups from day old to six weeks of age is presented in Table 7 and Fig 3. The weekly feed consumption increased in all groups. The feed consumption was highest in G 6 (1468 g) chicken and lowest in G4 (1316 g) chicken at first week of age. At second week of age, feed consumption was lowest in G1 (2422 g) chicken and highest in G6 (2724 g) chicken. At third week of age G2 (4500 g) and G5 (4500 g) had the highest feed consumption value and lowest was observed in G1 (4060 g). During fourth week of age highest feed consumption was in G 5 (6740 g) chicken and lowest in G 4 (5800 g) chicken. At fifth week of age feed consumption was lowest in G3 (9000 g) and G4 (9000 g) stressed chicken without supplementation of *W. somnifera* in feed. G2, G 5 and G6 chicken showed a higher of feed consumption value at fifth week of age (9200 g). During sixth week of age feed consumption was highest in G6 (12800 g) chicken and lowest in G4 (11100 g) chicken.

4.2.2 Feed Efficiency

The feed efficiency values between all the experimental groups are presented in Table 8 and Fig 4. The feed efficiency value was highest in G5 (3.2) chicken and lowest in G3 chicken (2.13) at first week. During second week the lowest feed efficiency value was in G2 (1.49) chicken and highest in G6 (1.98). At third week of age there was difference in feed efficiency value between groups and lowest feed efficiency value was in G 2 (1.87) chicken and highest in G5 (2.16) chicken. At fourth week of age the highest feed efficiency value was in G6 (1.92) and lowest in G3 (1.66). At fifth week of age the highest feed efficiency value was in G6 (3.6) and lowest in G4 chicken (2.45). During the sixth week of

Table 7. Weekly feed consumption of experimental groups (Mean, n=10)

Feed consumption (g)						
Weeks / Groups	1	2	3	4	5	6
G 1	1336	2422	4060	6250	9400	11800
G 2	1412	2684	4500	6500	9200	12000
G 3	1462	2704	4262	6000	9000	12600
G 4	1316	2600	4080	5800	9000	11100
G 5	1416	2692	4500	6740	9200	11200
G 6	1468	2724	4200	6240	9200	12800

G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Table 8. Weekly feed efficiency of experimental groups (Mean, n=10)

	Feed efficiency					
Groups \ Weeks	1	2	3	4	5	6
G 1	2.67	1.78	1.96	1.92	2.8	3.65
G 2	2.16	1.49	1.87	1.74	3.4	3.2
G 3	2.13	1.76	2.06	1.66	3.2	2.8
G 4	2.47	1.81	1.89	1.67	2.45	6.16
G 5	3.2	1.52	2.16	1.90	2.75	4.5
G 6	2.64	1.98	2.04	1.92	3.6	3.98

G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Figure 3. Weekly feed consumption of experimental groups (Mean, n=10)

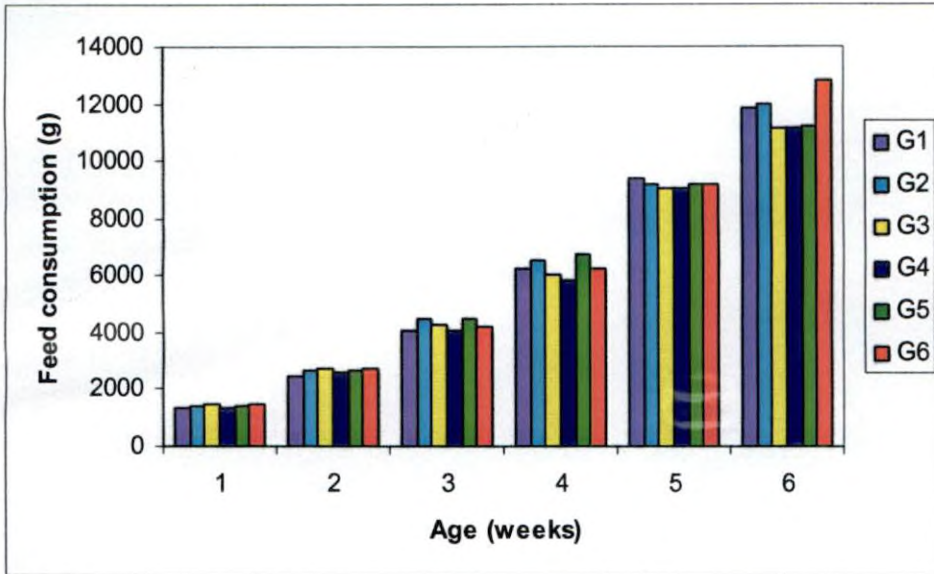
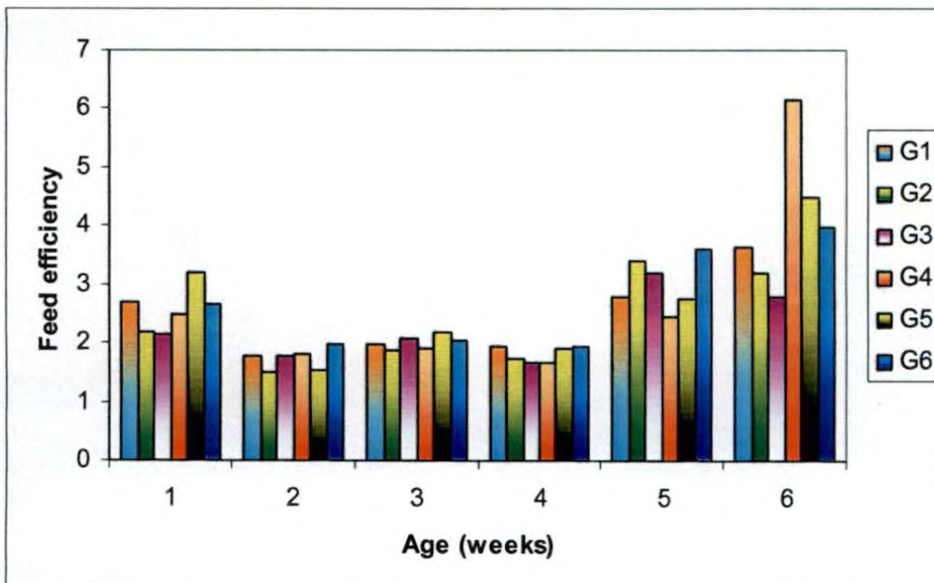


Figure 4. Weekly feed efficiency of experimental groups (Mean, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

age the highest feed efficiency value was observed in G4 (6.16) chicken and lowest in G3 (2.8) chicken.

4.3 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON SLAUGHTER PARAMETERS OF BROILER CHICKEN

4.3.1 Slaughter Weight

The slaughter weight (mean ± SE) between groups is presented in Table 9 and Fig 5a. The slaughter weights of G1, G2, G3, G4, G5 and G6 chicken were 2100 ± 72.65 g, 2000 ± 127.37 g, 1885 ± 119.27 g, 1740 ± 59.07 g, 1830 ± 76.81 g and 1900 ± 131.02 g respectively. There was no significant difference between groups on slaughter weight. The highest value was observed in G1 (2100 ± 72.65 g) and lowest in G4 (1740 ± 59.07 g).

4.3.2 Carcass Weight

The carcass weights (mean ± SE) between the groups are presented in Table 9 and Fig 5 b. The carcass weights of G1, G2, G3, G4, G5 and G6 chicken were 1255 ± 38.33 g, 1240 ± 76.30 g, 1135 ± 68.33 g, 1107.50 ± 37.65 g, 1112.50 ± 54.17 g and 1060.00 ± 83.60 g respectively. There was no significant difference between groups. The highest value was observed in G1 (1255 ± 38.33 g) chicken and lowest in G4 (1107.50 ± 37.65 g) chicken.

4.3.3 Giblet Weight

The giblet weights (mean ± S.E) between groups are presented in Table 9 and Fig 5c. The giblet weights in G1, G2, G3, G4, G5 and G6 chicken were 7.10±0.40, 6.28±0.27, 6.02±0.33, 6.31±0.34, 6.42±0.28 and 6.92±0.41 g per cent respectively. There was no significant difference between groups, but G1 chicken had higher value than other five groups. The G1 chicken had no significant

difference withG2, G3 and G4 chicken. G4 chicken did not have a significant difference with G5 and G6. Similar finding was observed between G5 and G6.

4.3.4 Weight of Bursa

The bursal weights (mean ± SE) between groups are presented in Table 9 and Fig 5d. The mean values of G1 to G6 chicken were 0.23±0.03, 0.18±0.01, 0.18±0.02, 0.11±0.01, 0.14±0.01 and 0.17±0.01 g per cent respectively. The G1 chicken had no significant difference with G2 and G3 chicken. G1 chicken had a significant ($P<0.05$) increase than G4 chicken. G4 chicken did not have a significant difference with G5 but had decrease in weight than G6. G5 and G6 chicken had no significant difference between the bursa weights.

4.3.5 Weight of Spleen

The spleen weights (mean ± SE) between groups are presented in Table 9 and Fig 5d. The mean value of spleen of G1, G2, G3, G4, G5 and G6 chicken were0.21±0.01, 0.20±0.01, 0.18±0.01, 0.18±0.02, 0.18±0.01 and 0.18±0.01 g per cent respectively. There was no significant difference in the weight of spleen between groups and the highest values were found in G1 chicken. G3, G4, G5 and G6 had similar value.

4.3.6 Weight of Adrenals

The weights of adrenals (mean ± SE) between groups are presented in Table 9 Fig 5d. The weight of adrenals of G1, G2, G3, G4, G5 and G6 chicken were 0.01±0.01, 0.02±0.01, 0.01±0.01, 0.03±0.01, 0.02±0.01 and 0.03±0.01 g per cent respectively. The G1 chicken had no significant difference with G2 and G3 chicken but was significantly ($P\leq0.05$) lower than G4 chicken. G2 and G3 chicken did not have any significant difference between them. G4 chicken had a

Table 9. Slaughter parameters of experimental groups (Mean ± SE, n=10)

Parameters / Groups	Slaughter weight (g) Mean± S.E. (n=10)	Carcass weight (g) Mean± S.E. (n=10)	Giblet (g per cent) Mean± S.E. (n=10)	Bursa (g per cent) Mean± S.E. (n=10)	Spleen (g per cent) Mean± S.E. (n=10)	Adrenals (g per cent) Mean± S.E. (n=10)
G 1	2100.00±72.65	1255.00±38.33	$7.10^{a}\pm0.40$	$0.23^{c}\pm0.03$	0.21±0.01	$0.01^{a}\pm0.01$
G 2	2000.00±127.37	1240.00±76.30	$6.28^{a}\pm0.27$	$0.18^{bc}\pm0.01$	0.20±0.01	$0.02^{ab}\pm0.01$
G 3	1885.00±119.27	1135.00±68.33	$6.02^{a}\pm0.33$	$0.18^{bc}\pm0.02$	0.18±0.01	$0.01^{a}\pm0.01$
G 4	1740.00±59.07	1107.50±37.65	$6.31^{a}\pm0.34$	$0.11^{a}\pm0.01$	0.18±0.02	$0.03^{d}\pm0.01$
G 5	1830.00±76.81	1112.50±54.17	$6.42^{a}\pm0.28$	$0.14^{ab}\pm0.01$	0.18±0.01	$0.02^{bc}\pm0.01$
G 6	1900.00±131.02	1060.00±83.60	$6.92^{a}\pm0.41$	$0.17^{bc}\pm0.01$	0.18±0.01	$0.03^{cd}\pm0.01$

G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

Figure 5 a. Slaughter parameters of experimental groups (Mean ± SE, n=10)

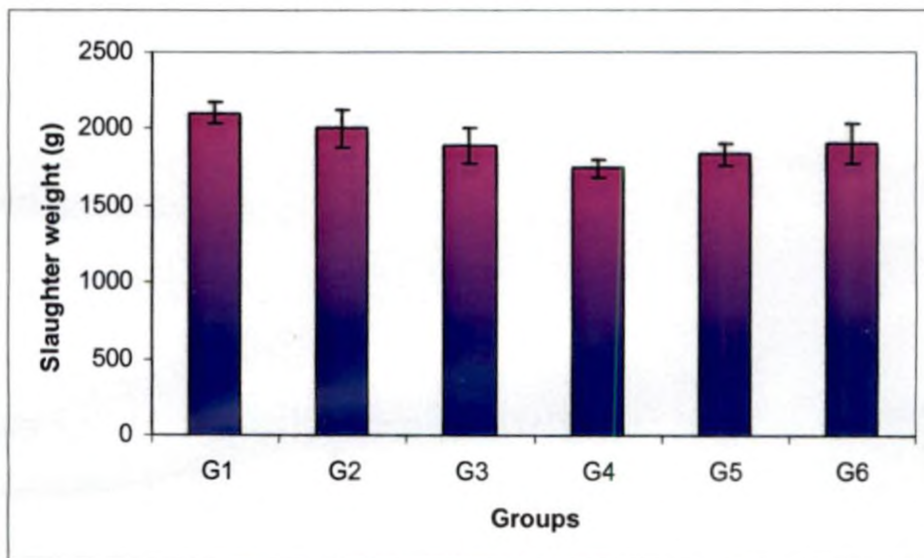
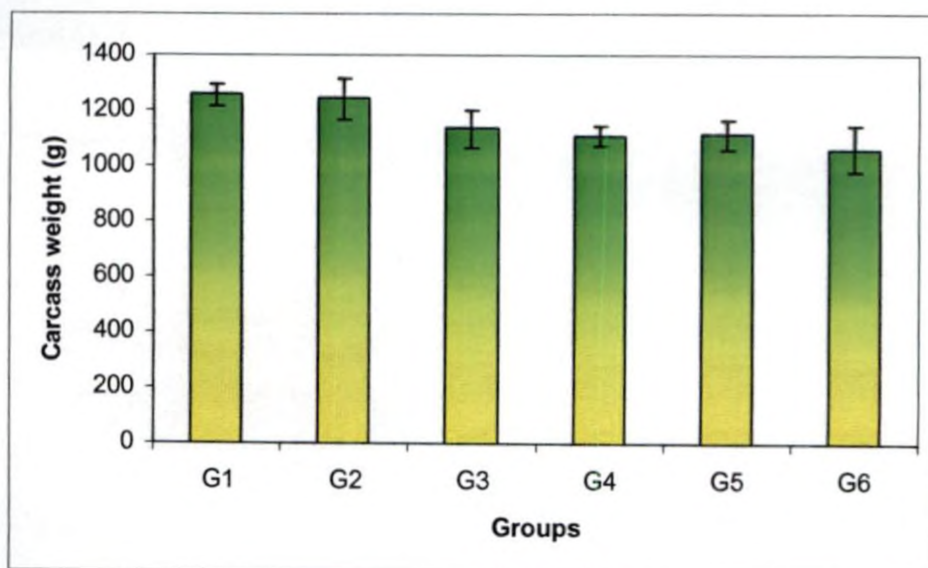


Figure 5 b. Slaughter parameters of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Figure 5 c. Slaughter parameters of experimental groups (Mean ± SE, n=10)

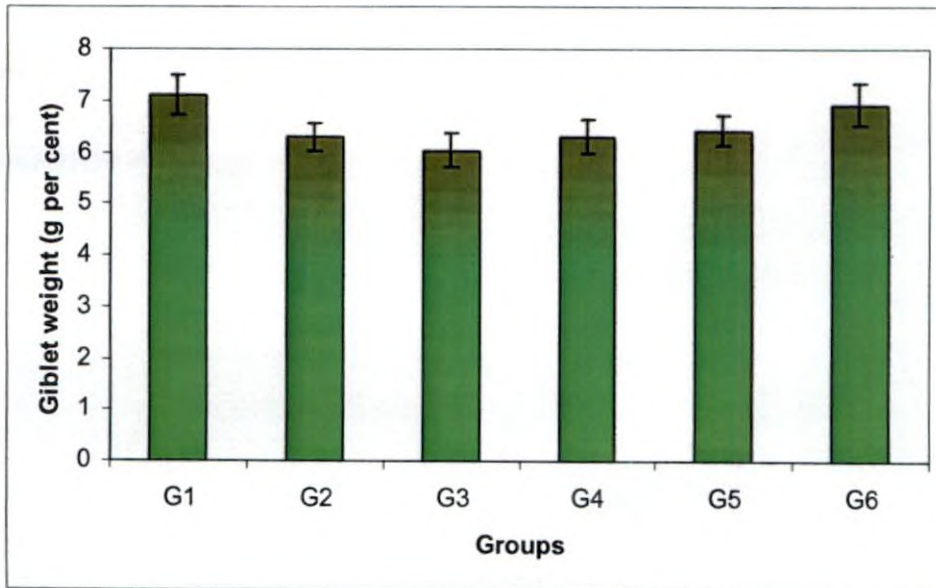
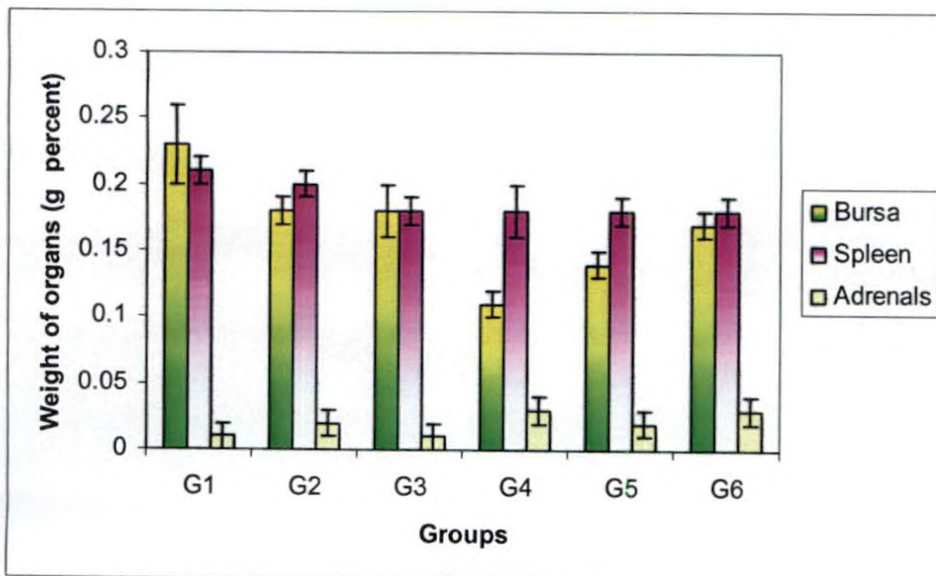


Figure 5 d. Slaughter parameters of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

significant ($P<0.05$) increase in adrenal weight compared to G5. G5 had no significant difference with G6 chicken.

4.4 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON HAEMATOLOGICAL PARAMETERS OF BROILER CHICKEN

4.4.1 Haemoglobin (Hb) Concentration

The Hb concentrations (mean ± SE) between all the experimental groups are given in Table 10 and Fig 6. Five days before stress there was no significant difference between the Hb levels of G1 to G6. G1 had no significant difference with G2 chicken, G3 chicken and G4 chicken one day before the induction of stress. There was no significant difference between G1, G4 and G5 chicken. G2 chicken showed a significant ($P<0.05$) decrease in Hb than G3 chicken one day before stress. G4, G5 chicken and G6 chicken did not show any significant difference between them.

One day after stress G1 (14.50 ± 0.99 g per cent) showed no significant difference with G2 and G3 chicken. There was a significant ($P<0.05$) decrease in Hb value of G4 (9.79 ± 0.66 g per cent) compared to G1 chicken. The Hb level in G4, G5 and G6 chicken did not show any significant difference among them. There was no significant difference in G1 to G6 chicken during the third, sixth, ninth, twelth and fifteenth day of stress.

The level of Hb between days within groups was compared with the levels obtained five days before induction of stress in each group. The G1 group (13.92 ± 1.28 g per cent) expressed a significant ($P<0.05$) increase in Hb concentration from the level obtained five day before stress (11.56 ± 0.61 g per cent). G1 chicken had a significant ($P<0.05$) increase in Hb level on day one (14.50 ± 0.99 g per cent), twelve (16.03 ± 0.92 g per cent) and fifteen days (19.07 ± 1.84 g per cent) of stress. There was no significant difference in Hb level on third, sixth and ninth day of stress.

In G2 chicken there was a significant (P<0.05) increase in Hb level on day six (15.14 ± 1.32 g per cent), nine (14.33 ± 1.42 g per cent), twelve (18.24 ± 1.28) and day fifteen days (19.07 ± 0.77 g per cent) of stress. There was no significant difference in Hb level one day before stress, one day and three days of stress.

In G3 chicken there was a significant (P<0.05) increase in Hb level one day (16.51 ± 1.07 g per cent) before stress, one day (16.84 ± 1.18 g per cent), three days (15.20 ± 1.44), nine (15.60 ± 1.83 g per cent), twelve (17.47 ± 1.30 g per cent) and fifteen days of (17.79 ± 1.13 g per cent) of stress. There was no significant difference in Hb level on the six day of stress.

G4 chicken showed a significant (P<0.05) increase one day (14.55 ± 0.90 g per cent) before stress, six (14.45 ± 1.05 g per cent), nine (15.29 ± 1.11 g per cent), twelve (15.79 ± 1.16 g per cent) and fifteen days (17.82 ± 0.85 g per cent) of stress. Hb level on one day (9.79 ± 0.66 g per cent) and three days (11.43 ± 1.22 g per cent) of stress did not show any significant difference with levels obtained before five days of stress.

G5 chicken showed a significant (P<0.05) increase in Hb level one day (13.62±0.95), nine (17.78 ± 1.36 g per cent), twelve (17.97 ± 1.78 g per cent) and fifteen days (20.43 ± 1.47 g per cent) of stress. There was no significant difference on first, third and sixth day of stress.

G6 chicken showed a significant (P<0.05) increase on nine (16.96 ± 1.01 g per cent), twelve (19.02 ± 1.74 g per cent) and fifteen days (20.57 ± 1.19 g per cent) of stress. There was no significant difference between one day before stress and one day, three and six days of stress.

4.4.2 Volume of Packed Red Cells (VPRC)

The mean ± S.E of VPRC between all the experimental groups are given in Table 11 and Fig 7. G1 chicken (35.00 ± 2.28 per cent) showed a significant (P<0.05) increase in VPRC than G2 (29.90 ± 0.92 per cent), G3 (27.70 ± 0.63 per

cent) and G4 (28.40 ± 1.13 per cent) chicken one day before stress. G2 and G3 chicken did not show any significant difference between them. G5 chicken (32.20 ± 1.03 per cent) showed a significant ($P<0.05$) increase than G4 chicken. G5 chicken showed a significant ($P<0.05$) increase than G6 chicken (25.10 ± 0.77 per cent), while G6 chicken showed no significant difference than G4 chicken.

One day after induction of stress there was no significant difference between G1, G2, and G4 chicken. There was a significant ($P<0.05$) decrease in G4 (26.40 per cent) than G5 (30.20 ± 1.65 per cent) chicken. There was no significant difference between G5 and G6 chicken and also between G4 and G6 chicken. There was no significant difference between groups at third, sixth, ninth and fifteenth day of stress.

On twelfth day of stress there was a significant ($P<0.05$) decrease in VPRC value in G1 (25.20 ± 0.96 per cent) chicken compared to G4 (29.30 ± 0.80 per cent) chicken. G1 chicken did not show any a significant difference with G2 chicken and G3 chicken. G2 and G3 chicken did not show any significant difference between them. There was no significant difference between G4, G5, and G6 chicken. Similar findings were observed between G5 and G6 and between G4 and G6 chicken. Among the groups there was no significant difference five day before stress and at three, six, nine, and fifteen days of stress.

VPRC values within groups between days were compared with the values obtained five days before the induction of stress in each group. In G1 chicken there was a significant ($P<0.05$) increase in VPRC value one day (35.00 ± 2.28 per cent) before stress, and rest of the values had no significant difference.

G2 chicken had a significant ($P<0.05$) decrease in VPRC value after three days (28.70 ± 1.30 per cent) of stress. There was no significant difference one day before stress, and on first, sixth days, ninth days, twelfth (26.50 ± 0.99 per cent) and fifteenth day of stress.

Table 10. Haemoglobin (Hb) of experimental groups (Mean ± SE, n=10)

	Hb (g per cent)							
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	11.56±0.61	13.92^{ab*}±1.28	14.5^{bc*}±0.99	11.85±1.14	13.32±1.14	14.70±1.51	16.03*±0.92	19.07*±1.84
G 2	10.45±1.12	11.75^{a}±1.09	12.31^{ab}± 1.15	14.39±1.37	15.14*±1.32	14.33*±1.42	18.24*±1.28	19.07*±0.77
G 3	11.21±0.87	16.51^{b} *±1.07	16.84^{c*}±1.18	15.20*±1.44	14.23±1.69	15.60*±1.83	17.47*±1.30	17.79*±1.13
G 4	9.99±0.59	14.55^{ab*}±0.90	9.79^{a} ±0.66	11.43±1.22	14.45*±1.05	15.29*±1.11	15.79*±1.16	17.82*±0.85
G 5	10.08±0.67	13.62^{ab*}±0.95	13.02^{ab}±1.44	11.05±0.47	11.77±0.68	17.78*±1.36	17.97*±1.78	20.43*±1.47
G6	12.62±1.50	11.89^{a} ±1.25	12.80^{ab}±1.22	13.43±2.00	13.20±0.83	16.90*±1.01	19.02*±1.74	20.57*±1.19

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.
Means bearing the same superscript in the column do not differ significantly ($P \geq 0.05$) between groups.
In a column if there is no superscript it means no significant difference ($P \geq 0.05$) among six treatments.
*denotes significant difference ($P<0.05$) within a row from five days before stress.

Table11. Volume of packed red cell (VPRC) of experimental groups (Mean ± SE, n=10)

VPRC (per cent)								
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	26.50±0.98	35.00^{*d} ±2.28	27.0^{a} ±1.03	28.20±1.95	25.00±0.83	27.20±2.40	25.20^{a} ±0.96	26.70±0.75
G 2	26.70±0.60	29.90^{bc} ±0.92	25.40^{a} ±0.79	28.70* ±1.30	27.70±1.12	26.70±0.88	26.50^{ab} ±0.99	27.50±0.91
G 3	28.30± 0.8	27.70^{ab} ±0.63	27.80^{ab} ±0.57	27.70±0.60	26.90±0.75	26.70±1.09	23.70^{a*} ±0.82	26.30±0.76
G 4	26.30±1.45	28.40^{ab} ±1.13	26.40^{a} ±1.15	26.10±0.67	27.20±1.40	25.80±1.16	29.30^{bc} ±0.80	27.50±0.93
G 5	30.50±1.74	32.20^{cd} ±1.03	30.20^{b} ±1.65	28.20±0.73	28.50±1.79	29.00±1.57	30.50^{c} ± 1.45	27.90±1.14
G6	27.70±1.08	25.10^{a*} ±0.77	27.90^{ab} ±0.59	31.70±1.86	26.90±1.57	27.90±0.99	29.30^{bc} ±1.40	26.60±0.60

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P≥0.05) between groups.

In a column if there is no superscript it means no significant difference (P≥0.05) among six treatments.

*denotes significant difference (P<0.05) within a row from five days before stress.

Figure 6. Haemoglobin (Hb) of experimental groups (Mean ± SE, n=10)

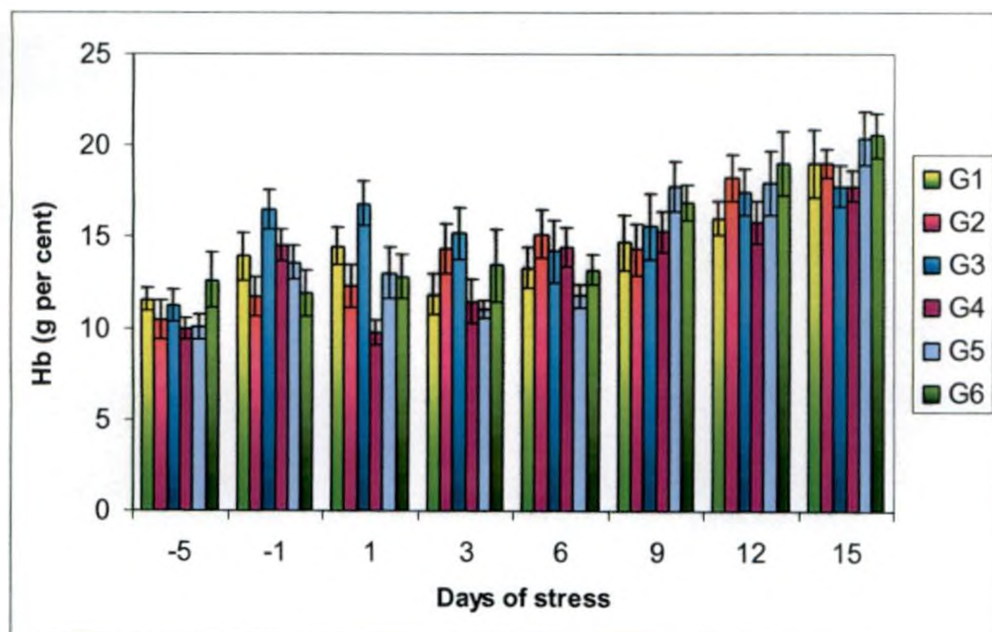
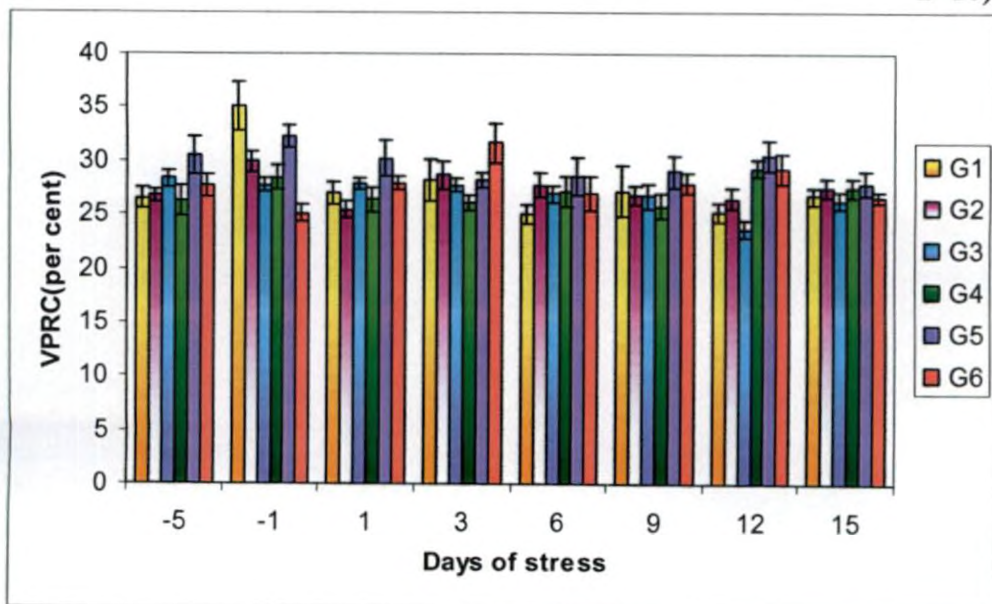


Figure 7. Volume of packed red cell (VPRC) of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

G3 chicken showed a significant (P<0.05) decrease in VPRC value on twelth day (23.70 ± 0.80 per cent) of stress. There was no significant difference one day before stress and on first, third, sixth, ninth and fifteenth day of stress.

G4 chicken did show any significant difference between days. A similar finding was also seen in G5 chicken. G6 chicken showed a significant (P<0.05) decrease one day (25.10 ± 0.77 per cent) before stress and no significant difference one day, three days, six days, nine days, twelve days and fifteen days of stress.

4.4.3 Total Erythrocyte Count (TEC)

The mean ± SE of TEC between all the experimental groups are given in Table 12 and Fig 8. G1 chicken did not show any significant difference with G2 and G3 chicken one day before stress. Similar findings were found between G2 and G3 chicken. G1 and G4 chicken also showed no significant difference between them. G4, G5 and G6 chicken also did not show any significant difference between them.

There was no significant difference in TEC between G1, G2 and G3 chicken after fifteen days of stress. There was a significant (P<0.05) decrease in G4 chicken (4.69 ± 0.34 $x10^6/\mu l$) compared to G1 chicken. A significant (P<0.05) increase was observed in G5 chicken (6.16 ± 0.42) than G4 chicken. G4 and G6 chicken (6.07 ± 0.23 $x10^6/\mu l$) also showed a similar significant (P<0.05) difference. G5 and G6 chicken did not show any significant difference between them. TEC did not show any significant difference between groups at five day before, one day, three, six, nine and twelve days of stress.

TEC within groups between days was compared with the values obtained five days before the induction of stress in each group. G1 chicken showed a significant (P<0.05) increase within groups one day (6.49 ± 0.33 $x10^6/\mu l$) before stress, one day (5.92 ± 0.33), three days (6.09 ± 0.26 $x10^6/\mu l$), six days (5.77 ±

0.51 x10^6/µl), nine days (7.01 ± 0.45), twelve days (6.46 ± 0.49) and fifteen days (6.47±0.35 x10^6/µl) of stress.

G2 chicken showed a significant ($P<0.05$) increase one day before (5.50 ± 0.21), one day (6.16 ± 0.09 x10^6/µl), three days (5.60 ± 0.25 x10^6/µl), six days (5.52 ± 0.47 x10^6/µl), nine days (6.28 ± 0.35), twelve days (6.74 ± 0.26 x10^6/µl) and fifteen days (6.35 ±0.24 x10^6/µl) of stress.

G3 chicken showed a significant ($P<0.05$) increase one day before (4.95 ± 0.41), one day (5.36 ± 0.52 x10^6/µl), three days (4.96 ± 0.41 x10^6/µl), six days (5.50 ± 0.40 x10^6/µl), nine days (6.59 ± 0.37 x10^6/µl), twelve days (6.08 ± 0.37 x10^6/µl) and fifteen days of stress (5.77 ± 0.28 x10^6/µl).

G4 chicken showed a significant ($P<0.05$) increase one day (5.93 ± 0.28 x10^6/µl), three days (6.14 ± 0.37 x10^6/µl), six days (5.64 ± 0.37 x10^6/µl), nine days (5.62 ± 0.48 x10^6/µl), and twelve days of stress (5.89 ± 0.32 x10^6/µl). There was no significant difference one day before and on fifteenth day of stress.

G5 chicken showed a significant ($P<0.05$) increase one day (5.49 ± 0.30), three days (5.74 ± 0.31 x10^6/µl), six days (5.65 ± 0.30 x10^6/µl), nine days (5.61 ± 0.40 x10^6/µl), twelve days (5.79 ± 0.22 x10^6/µl) and fifteen days (6.16 ± 0.42 x10^6/µl of stress). There was no significant difference one day before stress.

G6 chicken showed a significant ($P<0.05$) increase one day before stress (5.05 ± 0.45 x10^6/µl), one day (6.36 ± 0.67 x10^6/µl), three days (5.77 ± 0.56 x10^6/µl), six days (5.84 ± 0.56 x10^6/µl), nine days (6.88 ± 0.49 x10^6/µl), twelve days (6.38 ± 0.29 x10^6/µl) and fifteen days (6.07 ± 0.23 x10^6/µl) of stress.

4.4.4 Total Leukocyte Count (TLC)

The mean ± SE of TLC between all the experimental groups are given in Table 13 and Fig 9. Five days before stress there was a significant ($P<0.05$) decrease in G1 chicken (23.86 ± 0.46 x10^3/µl) compared to G2 (25.62 ± 0.32 x10^3/µl), G3 chicken (26.96 ± 0.43 x10^3/µl) and G4 (26.28 ± 0.52 x10^3/µl)

chicken. There was a significant ($P<0.05$) increase in G2 than G3 chicken and no significant difference between G2 and G4 and between G3 and G4 chicken. Chicken in G5 and G6 chicken did not show any significant difference between them.

G1 chicken (23.96 ± 0.43 x$10^3/\mu l$) showed a significant ($P<0.05$) decrease one day before stress from G2 (26.56 ± 0.25 x$10^3/\mu l$), G3 (27.59 ± 0.40 x$10^3/\mu l$) and G4 (25.81 ± 0.39 x$10^3/\mu l$) chicken. A similar finding was observed between G2 and G3 chicken but G3 had a significant ($P<0.05$) higher value than G4. There was no significant difference between G4, G5 and G6 chicken.

After one day of stress there was a significant ($P<0.05$) increase in G2 (27.16 ± 0.26 x$10^3/\mu l$), G3 (28.28 ± 0.34 x$10^3/\mu l$) and G4 chicken (25.86 ± 0.43 x$10^3/\mu l$) compared to G1 (24.61 ± 0.44 x$10^3/\mu l$). A similar finding was observed between G3 and G2 chicken. There was no significant difference between G4, G5 and G6 chicken.

Three days after stress G1 chicken (25.64 ± 0.45 x$10^3/\mu l$) had a significant ($P<0.05$) decrease compared to G2 (27.98 ± 0.25 x$10^3/\mu l$) and G3 chicken (29.54 ± 0.31 x$10^3/\mu l$). There was also a significant ($P<0.05$) decrease in G2 than G3 chicken. There was no significant difference between G1 and G4 chicken. There was also no significant difference between G4, G5 and G6 chicken.

After six days of stress G1 (27.39 ± 0.52 x$10^3/\mu l$) chicken had a significant ($P<0.05$) decrease than G2 (28.45 ± 0.21 x$10^3/\mu l$), G3 (31.32 ± 0.38 x$10^3/\mu l$) and a significant ($P<0.05$) increase than G4 chicken (25.08 ± 0.45 x$10^3/\mu l$). G3 chicken had a significant ($P<0.05$) increase than G2 and G4. There was no significant difference between G4, G5 and G6 chicken.

There was a significant ($P<0.05$) decrease in G1 (28.90 ± 0.48 x$10^3/\mu l$) chicken than G3 (32.24 ± 0.34 x$10^3/\mu l$) and an increase from G4 (24.62 ± 0.38) chicken after nine days of stress. There was no significant difference between G1 and G2 chicken. G2 chicken had a significant ($P<0.05$) decrease than G3 chicken

Table 12. Total erythrocyte count (TEC) of experimental groups (Mean ± SE, n=10)

Days / Groups	TEC ($x10^6/\mu l$)							
	-5	-1	1	3	6	9	12	15
G 1	4.45±0.27	6.49^{b*}±0.33	5.92^{*}±0.33	6.09^{*}±0.26	5.77^{*}±0.51	7.01^{*}±0.45	6.46^{*}±0.49	6.47^{b*}±0.35
G 2	4.83±0.28.	5.50^{ab*}±0.21	6.16^{*}±0.09	5.60^{*}±0.25	5.52^{*}±0.47	6.28^{*}±0.35	6.74^{*}±0.26	6.35^{b*}±0.24
G 3	3.89±0.42	4.95^{a*}±0.41	5.36^{*}±0.52	4.96^{*}±0.41	5.50^{*}±0.40	6.59^{*}±0.37	6.08^{*}±0.37	5.77^{b*}±0.28
G 4	4.73±0.26	5.63^{ab}±0.23	5.93^{*}±0.28	6.14^{*}±0.37	5.64^{*}±0.37	5.62^{*}±0.48	5.89^{*}±0.32	4.69^{a}±0.34
G 5	4.62±.0.31	5.16^{a}±0.23	5.49^{*}±0.30	5.74^{*}±0.31	5.65^{*}±0.30	5.61^{*}±0.40	5.79^{*}±0.22	6.16^{b*}±0.42
G6	4.09±0.35	5.05^{a*}±0.45	6.36^{*}±0.67	5.77^{*}±0.56	5.84^{*}±0.56	6.88^{*}±0.49	6.38^{*}±0.29	6.07^{b*}±0.23

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

* denotes significant difference (P<0.05) within a row from five days before stress.

Table 13. Total leukocyte count (TLC) of experimental groups (Mean ± SE, n=10)

Days / Groups	TLC (x10^3/ µl)							
	-5	-1	1	3	6	9	12	15
G 1	23.86^{a} ±0.46	23.96^{a} ±0.43	24.61^{a*} ±0.44	25.64^{a*} ±0.45	27.39^{b*} ±0.52	28.90^{b*} ±0.48	30.26^{c*} ±0.37	30.69^{c*} ±0.43
G 2	25.62^{b} ±0.32	26.56^{b} ±0.25	27.16^{c*} ±0.26	27.98^{b*} ±0.25	28.45^{c*} ±0.21	29.27^{b*} ±0.23	29.91^{c*} ±0.31	31.03^{c*} ±0.26
G 3	26.96^{c} ±0.43	27.59^{c*} ±0.40	28.28^{d*} ±0.34	29.54^{c*} ±0.31	31.32^{d*} ±0.38	32.24^{c*} ±0.34	32.96^{d*} ±0.32	33.46^{d*} ±0.28
G 4	26.28^{bc} ±0.52	25.81^{b*} ±0.39	25.86^{b*} ±0.43	25.48^{a*} ±0.45	25.08^{a*} ±0.45	24.62^{a*} ±0.38	24.06^{a*} ±0.33	24.02^{a*} ±0.27
G 5	26.20^{bc} ±0.22	26.12^{b} ±0.22	25.95^{b*} ±0.23	25.90^{a*} ±0.34	25.69^{a*} ±0.24	25.53^{a*} ±0.29	25.53^{b*} ±0.20	25.48^{b*} ±0.17
G 6	26.08^{bc} ±0.28	25.78^{b*} ±0.21	25.64^{b*} ±0.21	25.65^{a*} ±0.22	25.66^{a*} ±0.26	25.42^{a} *±0.23	25.27^{b*} ±0.19	24.99^{b*} ±0.23

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

* denotes significant difference (P<0.05) within a row from five days before stress.

Figure 8. Total erythrocyte count of experimental groups (Mean ± SE, n=10)

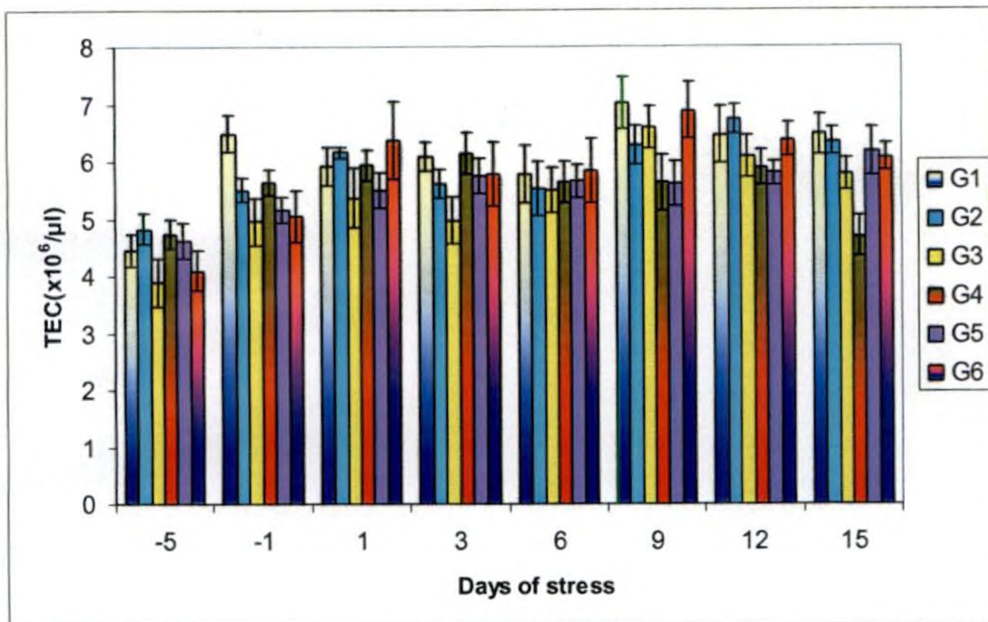
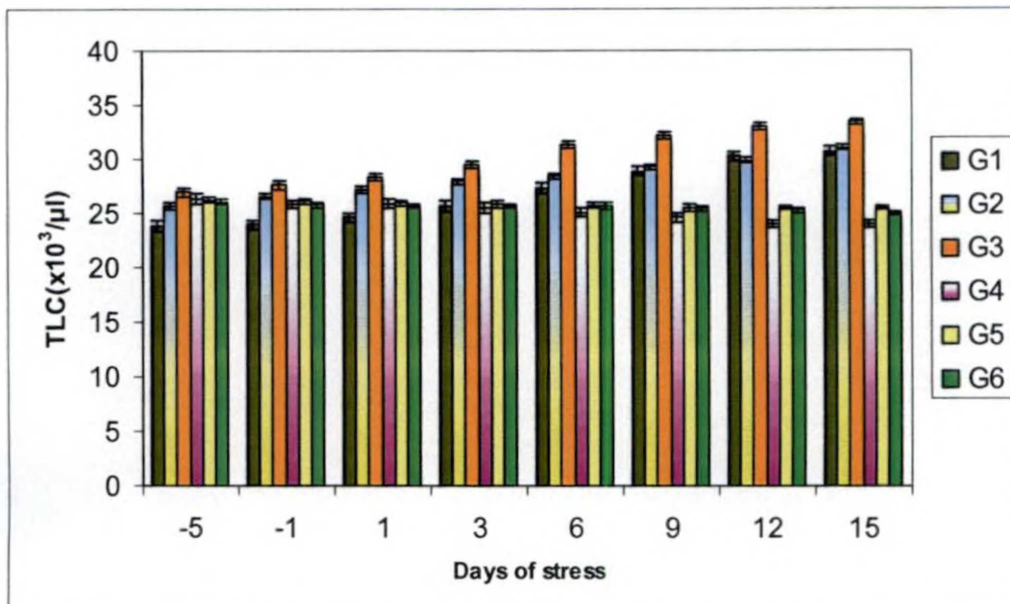


Figure 9. Total leukocyte count of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

and an increase than G4. There was no significant difference between G4, G5 chicken and G6 chicken.

At twelve days of stress G1 chicken (30.26 ± 0.37) showed a significant (P<0.05) increase than G3 (32.96 ± 0.32 $x10^3/\mu l$) and G4 (24.06 ± 0.33 $x10^3/\mu l$) chicken and no significant difference with G2 chicken. G2 showed a significant (P<0.05) decrease than G3 chicken but G4 had a significantly higher value than G3. There was no significant difference between G4, G5 and G6 chicken.

After fifteen days of stress G1 (30.69 ± 0.43) chicken showed a significant (P<0.05) decrease than G3 chicken (33.46 ± 0.28 $x10^3/\mu l$) and increase than G4 chicken (24.02 ± 0.27 $x10^3/\mu l$) but no significant difference with G2 chicken. There was a significant (P<0.05) increase in G3 compared to G4 chicken. G4 chicken showed a significant (P<0.05) decrease than G2 and G3 chicken. There was no significant difference between G5 and G6 chicken.

There was a significant (P<0.05) increase in TLC value between days within groups in all six groups after the induction of stress.

4.4.5 Mean Corpuscular Volume (MCV)

The mean ± SE of MCV between all the experimental groups are given in Table 14 and Fig 10. There was a significant (P<0.05) difference in MCV between groups on twelth and fifteenth day of stress. At twelve days of stress G1 (42.00 ± 4.52 fl) chicken had no significant difference in MCV value from G2 and G3 chicken but was significantly (P<0.05) lesser than G4 chicken (51.58 ± 3.98 fl). There was no significant difference between G2 and G3 chicken. There was no significant difference between G4, G5 and G6 chicken.

Fifteen days after stress there was no significant difference between G1, G2 and G3 chicken. G1 (42.80 ± 3.23 fl), G2 (44.13 ± 2.68 fl) and G3 (46.68± 2.89 fl) chicken showed a significant (P<0.05) decrease than G4 chicken (61.42 ± 4.65 fl). G4 chicken showed a significant (P<0.05) increase than G5 (49.16 ± 6.80

fl) and G6 chicken (44.49 ± 2.18 fl). G5 and G6 chicken showed no significant difference between groups. MCV value showed no significant difference one day before, three, six and nine days of stress.

MCV within groups between days were compared with the values obtained five days before the induction of stress in each group. G1 chicken showed a significant ($P<0.05$) decrease one day (47.00 ± 3.28), three days (46.97 ± 3.40 fl), nine days (40.53 ± 4.44 fl), twelve days (42.00 ± 4.52 fl) and fifteen days of stress (42.80 ± 3.23 fl). There was no significant difference one day before and after six days of stress.

G2 chicken showed a significant ($P<0.05$) decrease one day (41.25 ± 1.18), nine days (43.83 ± 2.88 fl), twelve days (40.11 ± 2.80 fl) and fifteen days (44.13 ± 2.68 fl) of stress and no significant difference one day before, three days and six days of stress.

G3 chicken showed a significant ($P<0.05$) decrease one day before (60.44 ± 6.30), one day (57.81 ± 7.00 fl), six days (51.04 ± 3.49 fl), nine days (42.02 ± 3.39 fl), twelve days (40.37 ± 2.83 fl) and fifteen days of stress (46.68 ± 2.89) and no significant difference on third day of stress.

G4 chicken showed a significant ($P<0.05$) decrease one day (45.54 ± 3.07 fl), three days (44.03 ± 2.83 fl) of stress and no significant difference was observed one day before, six days, nine days, twelve days and fifteen days of stress.

G5 chicken showed a significant ($P<0.05$) decrease on third day (50.31 ± 2.66 fl) and sixth day (51.50 ± 3.70 fl) of stress and no significant difference one day before, one day, nine days, twelve days and fifteen days of stress.

Chicken in G6 showed a significant ($P<0.05$) decrease one day before (53.85 ± 5.41) and one day (50.93 ± 7.65 fl), six days (51.20 ± 7.68 fl), nine days

(42.58 ± 3.45 fl), twelve days (46.02 ± 1.87 fl), and fifteen days of stress (44.49 ± 2.18 fl). There was no significant difference on the third day of stress.

4.4.6 Mean Corpuscular Haemoglobin (MCH)

The mean ± SE of MCH between all the experimental groups are given in Table 15 and Fig 11. There was a significant (P<0.05) decrease in G1 chicken (22.22 ± 2.59 pg) than G3 (37.47 ± 5.90 pg) chicken, one day before stress. But there was no significant difference between G2 and G4 chicken. G3 chicken had a significant (P<0.05) increase than G2 (21.84 ± 2.41pg) chicken. There was no significant difference between G4, G5 and G6 chicken.

One day of stress did not show any significant difference between G1, G2 and G4 chicken. G1 (25.13 ± 2.21 pg) chicken had a significantly (P<0.05) decreased value than G3 (36.27 ± 6.06) chicken. There was no significant difference between G4, G5 and G6 chicken.

After three days of stress G3 (35.97 ± 7.53 pg) chicken had a significant (P<0.05) increase than G1 chicken (19.45±1.56 pg). G1 did not show any significant difference between G2 and G4 chicken. G2 chicken had a significantly (P<0.05) decreased value compared to G3 chicken. G4 chicken had no significant difference with G5 and G6 chicken.

There was no significant difference in MCH between G1, G2 and G3 chicken on the fifteenth day of stress. G1 (29.68 ± 0.96 pg) showed a significant (P<0.05) decrease than G4 chicken (40.32± 4.22 pg). G4 had a significant (P<0.05) increase in value than G1, G2 (30.19 ± 1.06 pg), G3 (31.50 ± 2.43 pg) and G5 chicken (33.27±0.97 pg). There was no significant difference between G5 and G6 chicken. MCH value showed no significant difference on sixth, ninth, twelth day of stress between groups.

MCH within groups between days were compared with five days before stress in each group. G1 chicken showed a significant (P<0.05) decrease on third

Table 14. Mean corpuscular volume (MCV) of experimental groups (Mean ± SE, n=10)

	MCV (fl)							
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	61.40±4.16	54.64±3.47	47.00*±3.28	46.97*±3.40	47.61±6.00	40.53*±4.44	42.00^{ab*}±4.52	42.80^{a*}±3.23
G 2	57.09±3.75	54.72±1.73	41.25*±1.18	51.83±2.40	54.88±7.09	43.83*±2.88	40.11^{a*}±2.80	44.13^{a*}±2.68
G 3	79.66±8.34	60.44*±6.30	57.81*±7.00	61.56±7.82	51.04*±3.49	42.02*±3.39	40.37^{a*}±2.83	46.68^{a*}±2.89
G 4	56.11±2.27	51.35±2.96	45.54*±3.07	44.03*±2.83	49.69±3.29	49.89±5.81	51.58^{bc}±3.98	61.42^{b}±4.65
G 5	69.01±5.92	62.39±1.87	57.35±5.70	50.31*±2.66	51.50*±3.70	55.18±5.88	53.13^{c}±2.89	49.16^{a}±6.80
G 6	72.55±6.60	53.85*±5.41	50.93*±7.65	62.29±9.84	51.20*±7.68	42.58*±3.45	46.02^{abc*}±1.87	44.49^{a*}±2.18

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G 1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

* denotes significant difference (P<0.05) within a row from five days before stress.

Table 15. Mean corpuscular haemoglobin (MCH) of experimental groups (Mean ± SE, n=10)

MCH (pg) Mean ± S.E. (n=10)								
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	27.51±2.94	22.22^{a}±2.59	25.13^{a}±2.21	19.45^{a*}±1.56	24.47±2.42	21.95±2.66	27.25±3.81	29.68^{a}±0.96
G 2	22.13±2.47	21.84^{a}±2.41	20.20^{a}±2.14	25.85^{a}±2.20	32.95±7.28	24.94±4.52	27.63^{*}±2.56	30.19^{a*}±1.06
G 3	32.12±5.25	37.47^{b}±5.90	36.27^{b}±6.06	35.97^{b}±7.53	27.38±3.76	25.48±4.21	29.75±2.90	31.50^{a}±2.43
G 4	21.50±1.36	26.40^{ab}±2.33	17.21^{a}±1.77	19.15^{a}±2.10	27.41±3.57	27.89^{*}±1.60	27.51^{*}±2.67	40.32^{b*}±4.22
G 5	24.40±4.61	26.22^{ab}±1.03	23.57^{a}±1.84	19.78^{a}±1.41	21.36±1.82	35.47±5.88	31.95±3.87	33.27^{a}±0.98
G 6	32.46±3.63	26.21^{ab}±5.28	24.33^{a}±5.54	23.28^{a}±2.12	24.65^{*}±3.04	26.90±3.80	30.16±2.87	34.01^{ab}±1.74

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G 1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly ($P>0.05$) between groups.

In a column if there is no superscript it means no significant difference ($P>0.05$) among six treatments.

*denotes significant difference ($P<0.05$) within a row from five days before stress

Figure 10. Mean corpuscular volume of experimental groups (Mean ± SE, n=10)

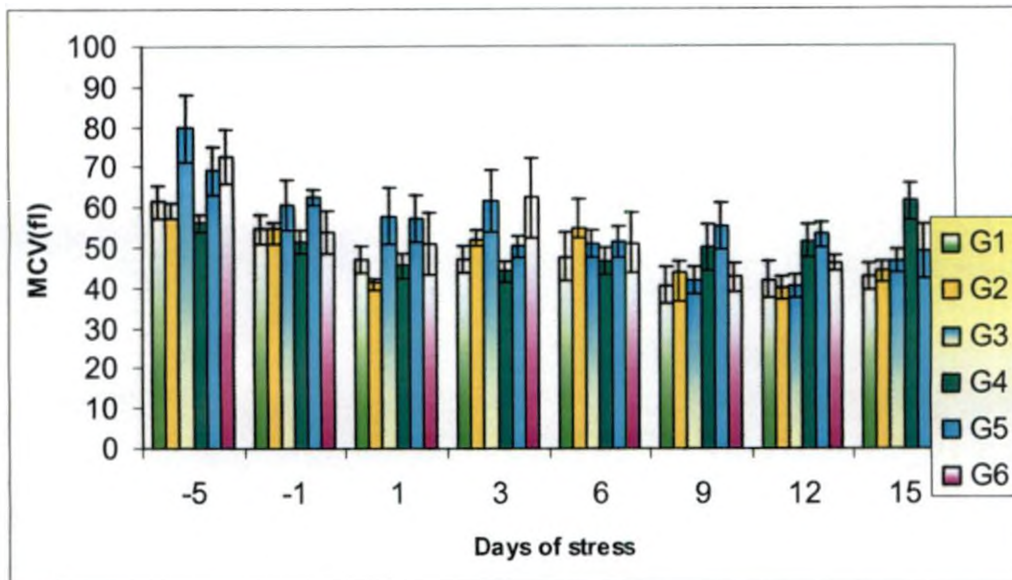
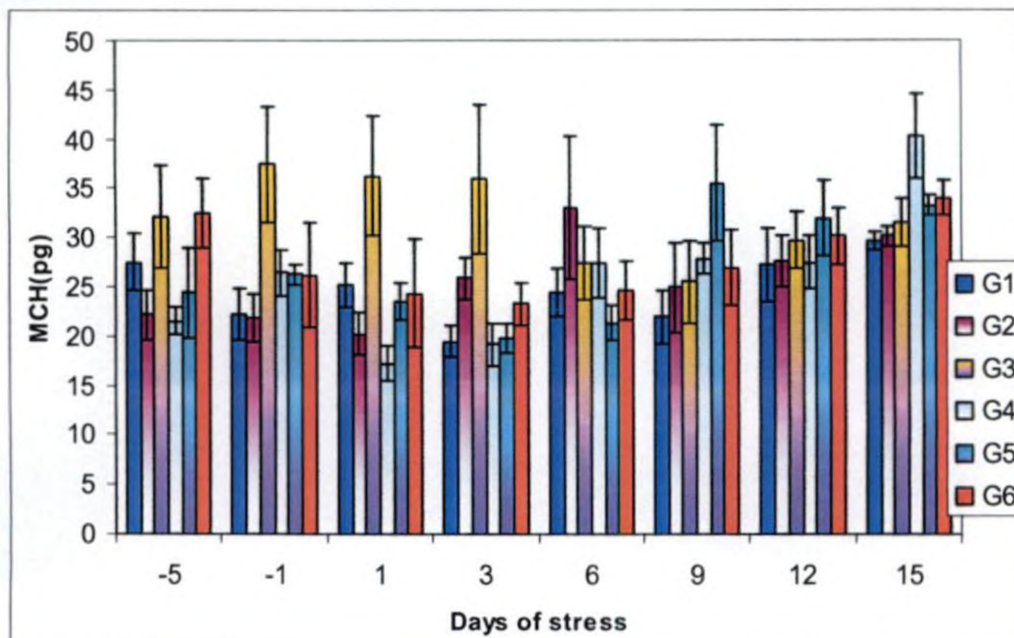


Figure 11. Mean corpuscular haemoglobin of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

day of stress (19.45± 1.56 pg) and no significant difference was observed one day before, one day, six days, nine days, twelve days and fifteen days of stress.

G2 chicken showed a significant (P<0.05) increase on twelth day (27.63± 2.56 pg) and fifteenth day (30.19 ± 1.06 pg) of stress. There was no significant difference one day before, one day, three days, six days and nine days of stress.

G3 chicken did not show any significant difference between days. G4 chicken showed a significant (P<0.05) increase on ninth day (27.89 ± 1.60 pg), twelth day (27.51 ± 2.67 pg) and fifteenth day (40.32 ± 4.22 pg) of stress and no significant difference was observed one day before, one day, three days and six days of stress.

G5 chicken did not show any significant difference in any of the days within groups.

G6 chicken showed a significant (P<0.05) decrease on sixth day (24.65 ± 3.04) of stress, but no significant difference was observed on other days.

4.4.7 Mean Corpuscular Haemoglobin Concentration (MCHC)

The mean ± SE of MCHC between all the experimental groups are given in Table 16 and Fig 12. There was a significant (P<0.05) decrease in G1 (40.56 ± 3.96 per cent) compared to G3 chicken (60.35 ± 4.62 per cent) and no significant difference between G1 and G4 chicken. There was also a significant (P<0.05) increase in G3 (60.35 ± 4.62 per cent) chicken than G2 (39.62 ± 3.72 per cent). G4 and G5 and G6 chicken did not show any significant difference between them.

After one day of stress there was no significant difference between G1, G2 and G3 chicken. A significant (P<0.05) increase in G1 (53.84 ± 3.33 per cent) chicken was observed compared to G4 chicken (37.79 ± 3.26 per cent). There was no significant difference among G4, G5 and G6 chicken.

MCHC within groups between days were compared with the values obtained five days before the induction of stress in each group. G1 chicken showed a significant ($P<0.05$) increase one day (53.84 ± 3.33), six days (53.69 ± 4.55 per cent), nine days (57.62 ± 6.88 per cent), twelve days (64.43 ± 4.24) and fifteen days (71.94 ± 4.22 per cent) of stress and no significant difference on one day before and after three days of stress.

G2 chicken showed a significant ($P<0.05$) increase one day (49.32 ± 5.33 per cent), three days (50.75 ± 5.12 per cent), six days (56.39 ± 6.55 per cent), nine days (54.70 ± 6.40 per cent), twelve days (69.08 ± 4.74 per cent) and fifteen days (70.74 ± 5.21 per cent) of stress. There was no significant difference on day one day before stress.

G3 chicken showed a significant ($P<0.05$) increase one day before (60.35 ± 4.62) and one day (61.21 ± 4.83 per cent), three days (55.46 ± 5.62 per cent), six days (52.85 ± 5.82 per cent), nine days (59.71 ± 7.32 per cent), twelve days (73.29 ± 3.92 per cent) and fifteen days (68.14 ± 4.63 per cent) of stress.

G4 chicken showed a significant ($P<0.05$) increase one day before (52.23 ± 4.03), six days (55.19 ± 5.92 per cent), nine days (60.85 ± 6.34 per cent), twelve days (54.19 ± 4.01 per cent) and fifteen days (65.62 ± 4.05 per cent) of stress and no significant difference on day one and three day of stress.

G5 chicken showed a significant ($P<0.05$) increase nine days (61.71 ± 4.42 per cent), twelve days (60.41 ± 6.74 per cent) and fifteen days (74.90 ± 6.42 per cent) of stress and no significant difference one day before, one day, three days and six days of stress.

G6 chicken showed a significant ($P<0.05$) increase on ninth day (61.22 ± 4.25), twelth day (66.08 ± 6.59 per cent) and fifteenth day (77.80 ± 5.04 per cent) of stress and no significant difference one day before, three and six days of stress.

4.4.8 Heterophil Lymphocyte Ratio (H:L)

The mean ± SE of H:L between all the experimental groups are given in Table 17 and Fig 13. After three days of stress there was significant (P<0.05) difference among G1 chicken (0.51 ± 0.01), G2 (0.49 ± 0.01) G3 (0.49 ± 0.01) and G4 chicken (0.53 ± 0.00). There was no significant difference among G4, G5 and G6 chicken.

On ninth day of stress there was a significant (P<0.05) increase in G1 chicken (0.59 ± 0.01) compared to G2 (0.51 ± 0.01) and G3 chicken (0.53 ± 0.01) but there was no significant difference between G1 and G4 chicken. There was a significant (P<0.05) decrease in G5 (0.58 ± 0.01) and G6 chicken (0.57 ± 0.01) compared to G4 chicken. There was no significant difference between G5 and G6 chicken.

On twelth day of stress G1 chicken (0.60 ± 0.01) showed a significant (P<0.05) increase than G3 (0.51 ± 0.01) and a decrease than G4 chicken (0.67 ± 0.01). G1 chicken showed no significant difference with G2 chicken. G2 and G3 chicken showed no significant difference between them. G4 chicken showed a significant (P<0.05) increase compared to G5 (0.58 ± 0.01) and G6 (0.60 ± 0.02) groups. G5 and G6 chicken did not show a significant difference between them.

On fifteenth day of stress G1 chicken (0.54 ± 0.01) showed a significant (P<0.05) increase than G3 (0.49 ± 0.01) and decrease than G4 (0.74 ± 0.02) chicken. There was no significant difference between G1 and G2 chicken. G2 (0.53 ± 0.01) showed a significant (P<0.05) increase than G3 chicken. G4 chicken showed a significant (P<0.05) increase than G5 (0.60 ± 0.01) and G6 (0.59 ± 0.01) chicken. G5 and G6 chicken did not show any significant difference between them. H:L had no significant difference one day before, one day and six days of stress.

H:L within groups between days were compared with values obtained five days before the induction of stress in each group. G1 chicken showed significant

(P<0.05) increase after three days (0.51 ± 0.01), six days (0.55 ± 0.01), twelve days (0.60 ± 0.01) and fifteen days (0.54 ± 0.01) of stress and no significant difference one day before and one day after stress.

G2 chicken showed a significant (P<0.05) increase on ninth day (0.51 ± 0.01), and twelth day (0.55 ± 0.01) day of stress and no significant difference one day before, one day, three days, six days and fifteen days of stress.

G3 chicken showed a significant (P<0.05) increase on third day (0.49 ± 0.01), sixth day (0.54 ± 0.01), ninth day (0.53 ± 0.01), twelth day (0.51 ± 0.01) and fifteenth day (0.49 ± 0.01) of stress, and no significant difference one day before and one day after stress.

G4 chicken showed a significant (P<0.05) increase three day (0.53 ± 0.01), six days (0.59 ± 0.01), nine days (0.62 ± 0.01), twelve day (0.67 ± 0.01) and fifteen days (0.74 ± 0.02) of stress and no significant difference one day before and one day after stress.

G5 chicken showed a significant (P<0.05) increase three days (0.53 ± 0.01), six days (0.56 ± 0.01), nine days (0.58 ± 0.01), twelve days (0.58 ± 0.01) and fifteen days (0.60 ± 0.01) of stress and no significant difference one day before and one day after stress.

G6 chicken showed a significant (P<0.05) increase after six days (0.55 ± 0.01), nine days (0.57 ± 0.01) twelve days (0.60 ± 0.02) and fifteen days (0.59 ± 0.01) of stress and no significant difference one day before, one day and three days of stress.

Table 16. Mean corpuscular haemoglobin concentration (MCHC) of experimental groups (Mean ± SE, n=10)

MCHC (per cent)								
Days / Groups	-5	-1	1	3	6.	9	12	15
G 1	44.33±3.21	40.56^{ab}±3.97	53.84^{bc*}±3.33	44.73±6.23	53.69*±4.55	57.62*±6.88	64.43*±4.24	71.94*±4.22
G 2	39.23±4.20	39.62^{a}±3.72	49.32^{abc*}±5.33	50.75*±5.12	56.39*±6.55	54.70*±6.40	69.08*±4.74	70.74*±5.21
G 3	39.74±3.00	60.35^{c*}±4.62	61.21^{c*}±4.83	55.46*±5.62	52.85*±5.82	59.71*±7.32	73.29*±3.92	68.14*±4.63
G 4	38.46±1.98	52.23^{bc*}±4.03	37.79^{a}±3.26	43.65±4.19	55.19±*5.92	60.85*±6.34	54.19*±4.01	65.62*±4.05
G 5	34.70±3.79	42.02^{ab}±2.24	45.58^{ab}±6.67	39.39±1.85	42.67±3.42	61.71*±4.42	60.41*±6.74	74.90*±6.42
G 6	46.21±6.04	47.10^{ab}±4.51	46.10^{ab}±4.48	43.39±6.48	49.42±2.59	61.22*±4.25	66.08*±6.59	77.80*±5.04

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

*denotes significant difference (P<0.05) within a row from five days before stress.

Table 17. Heterophil lymphocyte (H:L) ratio of experimental groups (Mean ± SE, n=10)

H:L								
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	0.49±0.01	0.48±0.01	0.48±0.01	0.51^{ab*}±0.01	0.55*±0.01	0.59^{bc*}±0.01	0.60^{b*}±0.01	0.54^{b*}±0.01
G 2	0.49±0.01	0.49±0.00	0.51±0.01	0.49^{a}±0.01	0.52±0.01	0.51^{a*}±0.01	0.55^{ab*}±0.01	0.53^{b}±0.01
G 3	0.49±0.01	0.48±0.00	0.48±0.00	0.49^{a*}±0.01	0.54*±0.01	0.53^{a*}±0.01	0.51^{a*}±0.01	0.49^{a*}±0.01
G 4	0.47±0.01	0.48±0.00	0.49±0.01	0.53^{b*}±0.01	0.59*±0.01	0.62^{c*}±0.01	0.67^{c*}±0:01	0.74^{d*}±0.02
G 5	0.49±0.01	0.49±0.01	0.51±0.01	0.53^{b*}±0.01	0.56*±0.01	0.58^{b*}±0.01	0.58^{b*}±0.01	0.60^{c*}±0.01
G 6	0.48±0.01	0.49±0.00	0.50±0.01	0.52^{ab}±0.01	0.55*±0.01	0.57^{b*}±0.01	0.60^{b*}±0.02	0.59^{c*}±0.01

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

*denotes significant difference (P<0.05) within a row from five days before stress.

Figure 12. Mean corpuscular haemoglobin concentration on of experimental groups (Mean ± SE, n=10)

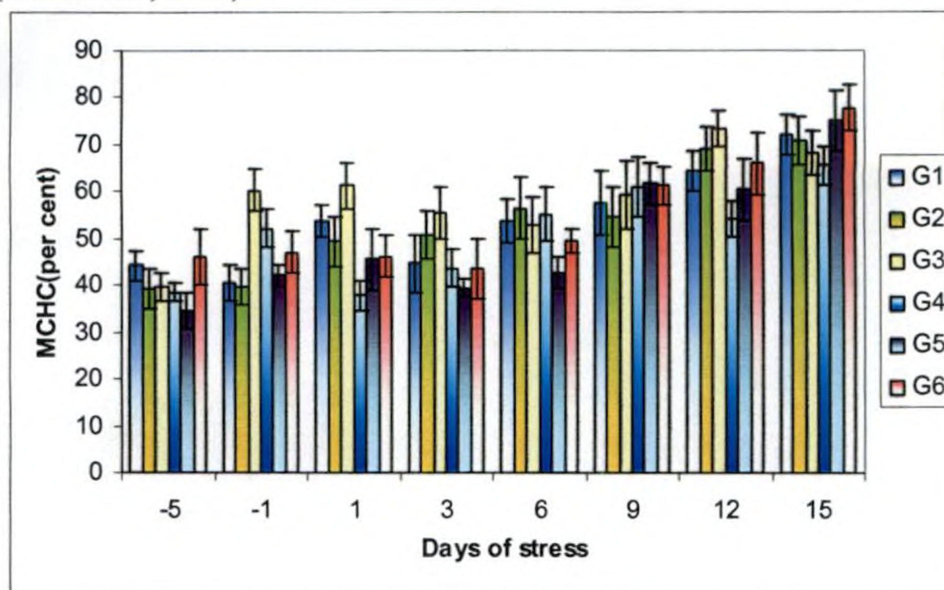
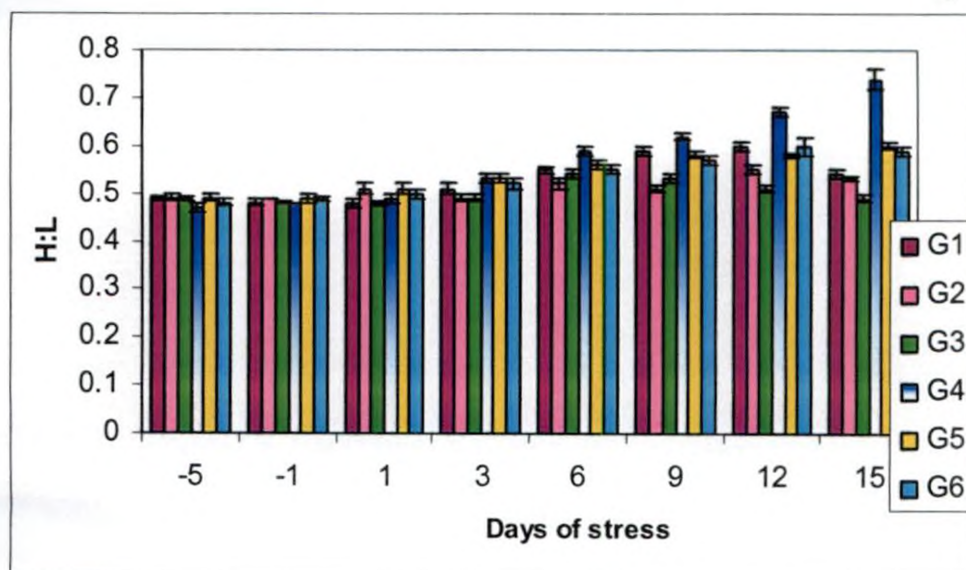


Figure 13. Heterophil lymphocyte ratio (H:L) of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

4.5 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON BLOOD BIOCHEMICAL PARAMETERS OF BROILER CHICKEN

4.5.1 Serum Protein Profile

4.5.1.1 Serum Total Protein

The mean ± SE of total protein between all the experimental groups are given in Table 18 and Fig 14. G1 chicken at five days before stress did not show any significant difference between G2 and G4 chicken. G3 (4.24 ± 0.28) chicken had a significantly ($P<0.05$) higher value than G1 (2.94 ± 0.13 g/dl), G2 (3.08 ± 0.20 g/dl) and G4 (3.31 ± 0.26 g/dl) chicken. Between G2 and G3 chicken had no significant difference could be observed. There was no significant difference between G4, G5 and G6 chicken.

There was no significant difference between G1, G2, G3 and G4 chicken, one day before stress. G6 (2.61 ± 0.07 g/dl) chicken had a significantly ($P<0.05$) lower value compared to G4 (3.35 ± 0.22 g/dl) and G5 (3.25 ± 0.14 g/dl) chicken. There was no significant difference between G4 and G5 chicken.

After six days of stress there was a significant ($P<0.05$) increase in G1 (3.93 ±0.18 g/dl) compared to G2 (3.59 ± 0.33 g/dl), G3 (3.37 ± 0.20 g/dl) and G4 (3.09 ± 0.17 g/dl) chicken. There was no significant difference between G2 and G3 chicken. G4, G5 and G6 chicken showed no significant difference between them.

At twelve days of stress G1 (3.82 ± 0.27 g/dl) chicken had a significant ($P<0.05$) increase than G2 (3.06 ± 0.11 g/dl) chicken and no significant difference with G3 and G4 chicken. There was no significant difference between G2 and G3 chicken and also between G4 and G5 chicken. But G6 (4.22 ± 0.29 g/dl) chicken had a significantly ($P<0.05$) higher value than G5 (3.86 ± 0.13 g/dl) chicken.

On fifteenth day of stress G1 chicken (4.36 ± 0.19 g/dl) showed a significant ($P<0.05$) increase than G3 (3.14 ± 0.13 g/dl) and G4 (3.63 ± 0.12 g/dl) chicken and no significant difference with G2 chicken. G2 (3.84 ± 0.27 g/dl) chicken also showed a significant ($P<0.05$) increase than G3 chicken. There was no significant difference between G4 and G5 and also between G4 and G6 chicken. G5 (3.90 ± 0.26 g/dl) chicken had a significant ($P<0.05$) increase than G6 (3.73 ± 0.22 g/dl) chicken.

Total protein between days within groups were compared with values obtained five days before the induction of stress in each group. G1 chicken showed a significant ($P<0.05$) increase one day (3.78 ± 0.20), three days (4.26 ± 0.31 g/dl), six days (3.93 ± 0.18 g/dl), twelve days (3.82 ± 0.27 g/dl) and fifteen days (4.36 ± 0.19 g/dl) of stress. There was no significant difference one day before and after nine days of stress.

G2 chicken showed a significant ($P<0.05$) increase one day (3.72 ± 0.18 g/dl), three days (4.74 ± 0.89 g/dl) and fifteen days (3.84 ± 0.27 g/dl) of stress and no significant difference one day before, six days, nine days and twelve days of stress.

G3 chicken showed a significant ($P<0.05$) increase one day before (3.27 ± 0.11 g/dl), one day (3.22 ± 0.23 g/dl), six days (3.37 ± 0.20 g/dl), nine days (3.11 ± 0.15 g/dl) and fifteen days (3.14 ± 0.13 g/dl) of stress and no significant difference three days and twelve days of stress.

G4 chicken showed a significant ($P<0.05$) increase one day (3.98 ± 0.25 g/dl), three days (3.99 ± 0.34 g/dl), twelve days (3.86 ± 0.13 g/dl) and fifteen days (3.63 ± 0.12 g/dl) of stress and no significant difference one day before, six days and nine days of stress.

G5 chicken showed a significant ($P<0.05$) increase one day (4.17 ± 0.17 g/dl), three days (4.09 ± 0.36 g/dl), nine days (3.74 ± 0.28 g/dl) and twelve days

(4.09 ± 0.21 g/dl) and fifteen day (3.90 ± 0.26 g/dl) of stress. There was no significant difference one day before, after six days and fifteen days of stress.

G6 chicken showed a significant (P<0.05) decrease one day before stress (2.61 ± 0.07 g/dl) after six days (3.01 ± 0.15 g/dl), nine days (3.50 ± 0.23 g/dl) and fifteen days (3.73 ± 0.22 g/dl) of stress. There was no significant difference one day, three days, and twelve days of stress.

4.5.1.2 Serum Albumin

The mean ± SE of serum albumin between all the experimental groups are given in Table 19 and Fig 15. Serum albumin of G1 (1.66 ± 0.04 g/dl) chicken one day before stress showed a significant (P<0.05) decrease than G2 (1.53 ± 0.03 g/dl) and G3 chicken (1.45 ± 0.04 g/dl) and no significant difference with G4. There was no significant difference between G2 and G3 chicken. There was no significant difference between G4, G5 and G6 chicken. One day after stress there was no significant difference between G1, G2 and G3. There was a significant (P<0.05) increase in value in G4 (1.74±0.01g/dl) compared to G2 (1.51 ± 0.03 g/dl) and G3 (1.42 ± 0.05 g/dl) chicken. There was no significant difference among G4 (1.74 ± 0.01 g/dl), G5 and G6 chicken.

Serum albumin levels between days within group were compared with values obtained five days before the induction of stress in each group. G1 chicken showed a significant (P<0.05) increase one day before (1.66 ± 0.44 g/dl), one day (1.57 ± 0.06 g/dl), three days (1.58 ± 0.09), six days (1.59 ± 0.05 g/dl) and nine days (1.72 ± 0.10 g/dl) of stress and no significant difference after twelve days and fifteen days of stress.

G2 chicken showed a significant (P<0.05) decrease on twelth day (1.43 ± 0.02 g/dl) of stress and no significant difference one day before, one day, three days, six days, nine days and fifteen days of stress.

Table 18. Serum total protein of experimental groups (Mean ± SE, n=10)

Total protein(g/dl)								
Days / Groups	5	-1	1	3	6	9	12	15
G 1	$2.94^{a} \pm 0.13$	$3.24^{b} \pm 0.23$	$3.78^{*} \pm 0.20$	$4.26^{*} \pm 0.31$	$3.93^{c*} \pm 0.18$	3.45 ± 0.22	$3.82^{bc*} \pm 0.27$	$4.36^{c*} \pm 0.19$
G 2	$3.08^{a} \pm 0.20$	$3.20^{b} \pm 0.12$	$3.72^{*} \pm 0.18$	$4.74^{*} \pm 0.39$	$3.59^{ab} \pm 0.33$	3.32 ± 0.15	$3.06^{a} \pm 0.11$	$3.84^{bc*} \pm 0.27$
G 3	$4.24^{c} \pm 0.28$	$3.27^{b*} \pm 0.11$	$3.22^{*} \pm 0.23$	4.10 ± 0.33	$3.37^{ab*} \pm 0.20$	$3.11^{*} \pm 0.15$	$3.53^{ab} \pm 0.20$	$3.14^{a*} \pm 0.13$
G 4	$3.31^{ab} \pm 0.26$	$3.35^{b} \pm 0.22$	$3.98^{*} \pm 0.25$	$3.99^{*} \pm 0.34$	$3.09^{a} \pm 0.17$	$3.31^{*} \pm 0.17$	$3.86^{bc*} \pm 0.13$	$3.63^{ab*} \pm 0.12$
G 5	$3.35^{ab} \pm 0.19$	$3.25^{b} \pm 0.14$	$4.17^{*} \pm 0.17$	$4.09^{*} \pm 0.36$	$3.16^{a} \pm 0.12$	$3.74^{*} \pm 0.28$	$4.09^{bc*} \pm 0.21$	$3.90^{bc} \pm 0.26$
G 6	$3.93^{bc} \pm 0.22$	$2.61^{a*} \pm 0.07$	3.88 ± 0.30	4.01 ± 0.26	$3.01^{a*} \pm 0.15$	$3.50^{*} \pm 0.23$	$4.22^{a} \pm 0.29$	$3.73^{a*} \pm 0.22$

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

*denotes significant difference (P<0.05) within a row from five days before stress.

Table 19. Serum albumin of experimental groups (Mean ± SE, n=10)

	Serum albumin (g/dl)							
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	1.47±0.04	1.66^{c*}±0.04	1.57^{ab*}±0.06	1.58^{*}±0.09	1.59^{*}±0.05	1.72^{*}±0.10	1.44±0.02	1.57±0.09
G 2	1.59±0.04	1.53^{ab}±0.03	1.51^{a}±0.03	1.61±0.03	1.60±0.04	1.62±0.03	1.43^{*}±0.02	1.50±0.12
G 3	1.64±0.10	1.45^{a*}±0.04	1.42^{a*}±0.05	1.65±0.06	1.53±0.05	1.54±0.06	1.42^{*}±0.03	1.43^{*}±0.04
G 4	1.63±0.05	1.56^{bc}±0.03	1.74^{bc}±0.01	1.54±0.05	1.68±0.06	1.51±0.07	1.54±0.07	1.59±0.08
G 5	1.54±0.02	1.57^{bc}±0.03	1.80^{c*}±0.09	1.60±0.04	1.59±0.07	1.45±0.05	1.51±0.10	1.65±0.06
G 6	1.62±0.06	1.57^{bc}±0.02	1.59^{abc}±0.06	1.53±0.06	1.49±0.08	1.47±0.07	1.51±0.05	1.58±0.05

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

*denotes significant difference (P<0.05) within a row from five days before stress.

Figure 14. Serum total protein of experimental groups (Mean ± SE, n=10)

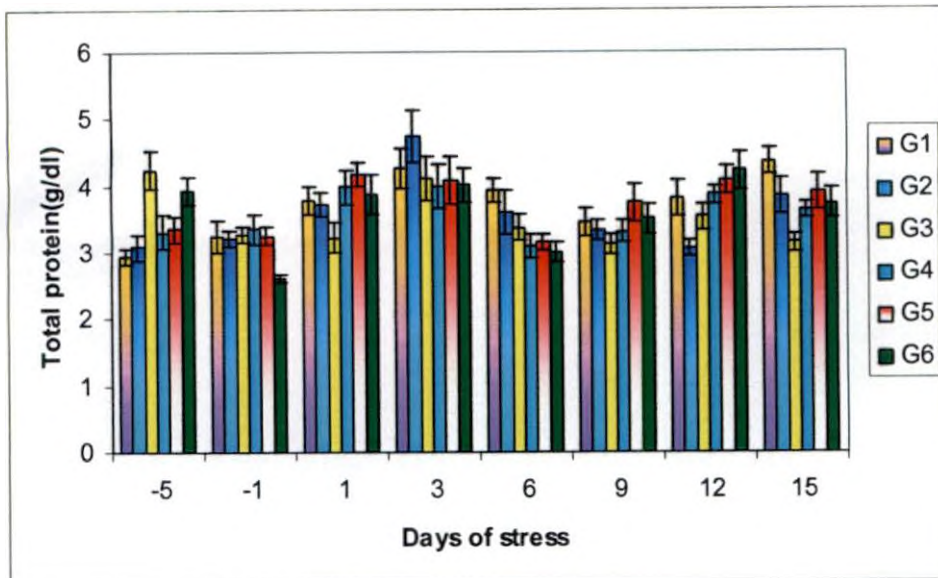
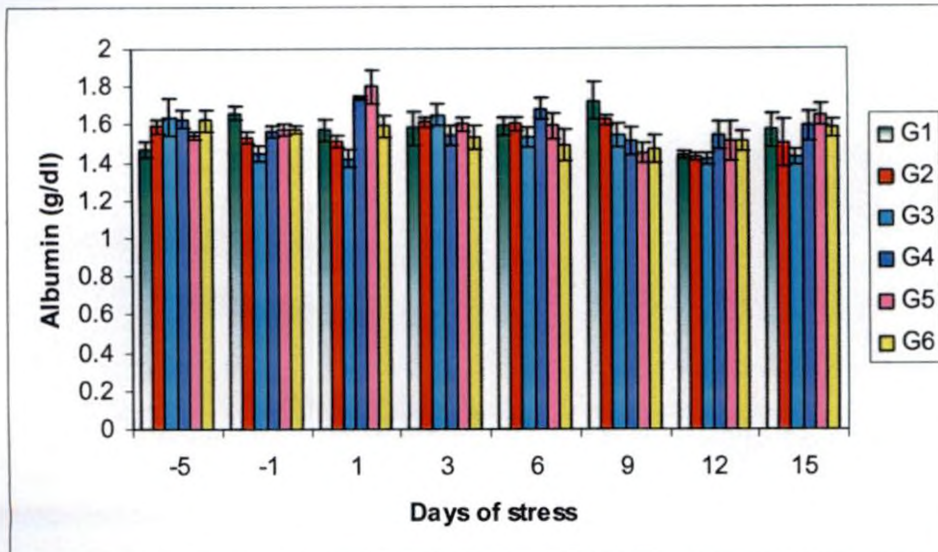


Figure 15. Serum albumin of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

G3 chicken showed a significant ($P<0.05$) decrease one day before (1.45 ± 0.04), one day (1.42 ± 0.05 g/dl), twelve days (1.42 ± 0.03 g/dl) and fifteen days (1.43 ± 0.04 g/dl) of stress. There was no significant difference after three, six and nine days of stress.

G4 chicken showed no significant difference between any days. G5 chicken showed a significant ($P<0.05$) increase one day (1.80 ± 0.09 g/dl) after stress and no significant difference one day before, three days, six days, nine days twelve days and fifteen days of stress.

G6 chicken showed no significant difference one day before stress, one day, three days, six days, nine days, twelve days and fifteen days of stress.

4.5.1.3 Serum Globulin

The mean ± SE of serum globulin between all the experimental groups are given in Table 20 and Fig 16. G1 (1.43 ± 0.11 g/dl) chicken expressed a significant ($P<0.05$) decrease in globulin value than G3 (2.60 ± 0.24 g/dl) chicken five days before stress. G2 (1.66 ± 0.15 g/dl) had a significant ($P<0.05$) decrease in globulin value than G3 (2.60 ± 0.24 g/dl) chicken. There was no significant difference among G1, G2 and G4. There was also no significant difference among G4, G5 chicken and G6 chicken.

One day before stress there was no significant difference among G1, G2, G3 and G4 chicken. There was also no significant difference between G4 and G5 chicken but G4 (1.77 ± 0.24 g/dl) and G5 (1.72 ± 0.13 g/dl) chicken had a significant ($P<0.05$) increase value than G6 (1.03 ± 0.09 g/dl) chicken.

On sixth day of stress, there was no significant difference among G1, G2 and G3 chicken. There was a significant ($P<0.05$) increase in globulin in G1 (2.40± 0.22 g/dl) compared to G4 (1.50 ± 0.15 g/dl) chicken. There was also no significant difference among G4, G5 and G6 chicken.

After nine days of stress, there was no significant difference among G1, G2 and G3. No significant difference was observed between G4, G5 and G6 chicken.

After twelve days of stress, no significant difference could be shown among G1, G3 and G4 chicken. Similarly no significant difference was observed between G2 and G3 chicken. There was a significant ($P<0.05$) increase in G1 (2.37 ± 0.27 g/dl) compared to G2 (1.63 ± 0.12 g/dl) chicken. There was no significant difference in globulin value among G4, G5 and G6 chicken.

At fifteen days of stress there was a significant ($P<0.05$) increase in G1 (2.58 ± 0.15 g/dl) and G2 (2.34 ± 0.23 g/dl) compared to G3 (1.71 ± 0.11 g/dl) chicken. There was no significant difference among G1 and G2 and G4 chicken. There was also no significant difference between G4, G5 and G6 chicken.

Serum globulin levels between days within groups were compared with values obtained five days before the induction of stress in each group. G1 chicken had a significant ($P<0.05$) increase one day before stress (1.73 ± 0.15 g/dl), one day (2.20 ± 0.19 g/dl), three days (2.19 ± 0.33 g/dl), six days (2.40 ± 0.22 g/dl), twelve days (2.37 ± 0.27 g/dl) and fifteen days (2.58 ± 0.15 g/dl) of stress and no significant difference in nine days of stress.

G2 chicken had a significant ($P<0.05$) increase one day (2.19 ± 0.16), three days (3.05 ± 0.35), six days (2.03 ± 0.28) and fifteen days (2.34 ± 0.23) of stress and no significant difference one day before, nine days and twelve days of stress.

G3 chicken had a significant ($P<0.05$) decrease one day before (1.81 ± 0.14 g/dl), one day (1.75 ± 0.21 g/dl), six days (1.83 ± 0.20 g/dl), nine days (1.57 ± 0.15 g/dl) and fifteen days (1.71 ± 0.11 g/dl) of stress and no significant difference on third and twelth day of stress.

G4 chicken had a significant (P<0.05) increase one day (2.23 ± 0.27 g/dl), three days (2.67 ± 0.35 g/dl), nine days (2.22 ± 0.14 g/dl), and twelve days (2.32 ± 0.10 g/dl) of stress and no significant difference one day before, six days and fifteen days of stress.

G5 chicken had a significant (P<0.05) increase three days (2.38 ± 0.32 g/dl), nine days (2.68 ± 0.21 g/dl) and twelve days (2.57 ± 0.14 g/dl) of stress and no significant difference one day before, one day, six days and fifteen days of stress.

G6 chicken had a significant (P<0.05) decrease one day before (1.03 ± 0.09 g/dl), six days (1.51 ± 0.13 g/dl) of stress and an increase (P<0.05) one day (2.73 ± 0.37 g/dl), three days (2.20 ± 0.23 g/dl), and twelve days (2.59 ± 0.22 g/dl) of stress and no significant difference on nine and fifteen days of stress.

4.5.1.4 Serum Albumin Globulin Ratio (A:G)

The mean ± SE of A:G between all the experimental groups are given in Table 21 and Fig 17. After five days before stress G1 (1.08 ± 0.09) chicken had a significant (P<0.05) increase than G3 (0.69 ± 0.08) chicken. There was no significant difference among G1, G2 and G4 chicken. G2 (1.01 ± 0.08) chicken had a significant (P<0.05) increase than G3 chicken. There was no significant difference among G4, G5 and G6 chicken.

One day before stress there was no significant difference among G1, G2, G3 and G4 chicken. There was no significant difference between G4 and G5 chicken but G6 (1.70 ± 0.26) chicken had a significantly (P<0.05) higher value than G4 (1.04 ± 0.13) and G5 (0.98 ± 0.09) chicken.

Six day after stress there was no significant difference between G1, G2, G1 and G3 chicken. G4 (1.26 ± 0.17) chicken had a significant (P<0.05) increase than G1 (0.72 ± 0.08) chicken. There was no significant difference among G4, G5 and G6 chicken.

There was also no significant difference between G1, G2 and G3 chicken on ninth day of stress. But there was a significant ($P<0.05$) increase in G1 (1.07 ± 0.14), G2 (1.06 ± 0.12), G3 (1.10 ± 0.14) than G4 (0.73 ± 0.09) chicken. There was no significant difference among G4, G5 and G6 chicken.

After twelve days of stress there was no significant difference among G1, G3 and G4 chicken. A similar finding was also found between G2 and G3 chicken. G2 (0.92 ± 0.07) chicken had a significant ($P<0.05$) increase than G1 (0.69 ± 0.08) and G4 (0.67 ± 0.04) chicken. There was no significant difference among G4, G5 (0.59 ± 0.02) and G6 chicken. After 15 days of stress, there was no significant difference among any of the group.

Serum A: G between days within groups was compared with values obtained five day before the induction of stress in each group. G1 chicken had a significant ($P<0.05$) decrease A:G one day (0.77 ± 0.09), six days (0.72 ± 0.08), twelve days (0.69 ± 0.08) and fifteen days (0.63 ± 0.06) of stress and no significant difference one day before, after three days and nine days

G2 chicken had a significant ($P<0.05$) decrease one day (0.72 ± 0.05), three days (0.65 ± 0.12) and fifteen days (0.70 ± 0.07) of stress and no significant difference one day before, six days, nine days and twelve days of stress.

G3 chicken had a significant ($P<0.05$) increase one day before (0.85 ± 0.08), after one day (0.94 ± 0.14), six days (0.91 ± 0.00), nine days (1.10 ± 0.14) and fifteen days (0.86 ± 0.05) of stress and no significant difference after three and twelve days of stress.

G4 chicken had a significant ($P<0.05$) decrease after twelve days (0.67 ± 0.04) of stress and no significant difference one day before, one day, three days, six days, nine days and fifteen days of stress.

Table 20. Serum globulin of experimental groups (Mean ± SE, n=10)

Serum globulin (g/dl)								
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	1.43^{a} ±0.11	1.73^{b*}±0.15	2.20*±0.19	2.19*±0.33	2.40^{b*}±0.22	1.82^{ab} ±0.20	2.37^{b*}±0.27	2.58^{b*}±0.15
G 2	1.66^{ab} ±0.15	1.66^{b} ±0.13	2.19*±0.16	3.05*±0.35	2.03^{ab*}±0.28	1.69^{ab} ±0.17	1.63^{a} ±0.12	2.34^{b*}±0.23
G 3	2.60^{c} ±0.24	1.81^{b*}±0.14	1.75*±0.21	2.44±0.32	1.83^{ab*}±0.20	1.57^{a*}±0.15	2.09^{ab} ±0.20	1.71^{a*}±0.11
G 4	1.84^{ab} ±0.24	1.77^{b}±0.24	2.23*±0.27	2.67*±0.35	1.50^{a} ±0.15	2.22^{bc*}±0.14	2.32^{b*}±0.10	2.14^{ab} ±0.08
G 5	1.80^{ab} ±0.19	1.72^{b} ±0.13	2.01±0.28	2.38*±0.32	1.57^{a}±0.12	2.68^{c*}±0.21	2.57^{b*}±0.14	2.19^{ab} ±0.28
G 6	2.28^{bc} ±0.22	1.03^{a*}±0.09	2.73*±0.37	2.20*±0.23	1.51^{a*}±0.13	2.42^{c}±0.17	2.59^{b*}±0.22	2.04^{ab} ±0.18

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.
Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.
In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.
*denotes significant difference (P<0.05) within a row from five days before stress.

Table 21. Albumin globulin ratio (A:G) of experimental groups (Mean ± SE, n=10)

A:G								
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	$1.08^{c}\pm0.09$	$1.02^{a}\pm0.08$	$0.77^{*}\pm0.09$	0.86 ± 0.12	$0.72^{a*}\pm0.08$	$1.07^{b}\pm0.14$	$0.69^{a*}\pm0.08$	$0.63^{*}\pm0.06$
G 2	$1.01^{bc}\pm0.08$	$0.97^{a}\pm0.07$	$0.72^{*}\pm0.05$	$0.65^{*}\pm0.12$	$0.90^{ab}\pm0.10$	$1.06^{b}\pm0.12$	$0.92^{b}\pm0.07$	$0.70^{*}\pm0.07$
G 3	$0.69^{a}\pm0.08$	$0.85^{a*}\pm0.08$	$0.94^{*}\pm0.14$	0.81 ± 0.12	$0.91^{ab*}\pm0.09$	$1.10^{b*}\pm0.14$	$0.74^{ab}\pm0.07$	$0.86^{*}\pm0.05$
G 4	$1.01^{bc}\pm0.10$	$1.04^{a}\pm0.13$	0.92 ± 0.14	0.70 ± 0.11	$1.26^{b}\pm0.17$	$0.73^{a}\pm0.09$	$0.67^{a*}\pm0.04$	0.75 ± 0.05
G 5	$0.94^{abc}\pm0.09$	$0.98^{a}\pm0.09$	1.06 ± 0.14	0.89 ± 0.19	$1.09^{b}\pm0.11$	$0.57^{a*}\pm0.04$	$0.59^{a*}\pm0.02$	0.83 ± 0.07
G 6	$0.77^{ab}\pm0.02$	$1.70^{b*}\pm0.26$	0.69 ± 0.10	0.76 ± 0.09	$1.05^{ab}\pm0.10$	$0.63^{a}\pm0.06$	$0.61^{a*}\pm0.04$	0.81 ± 0.05

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

* denotes significant difference (P<0.05) within a row from five days before stress.

Figure 16. Serum globulin of experimental groups (Mean ± SE, n=10)

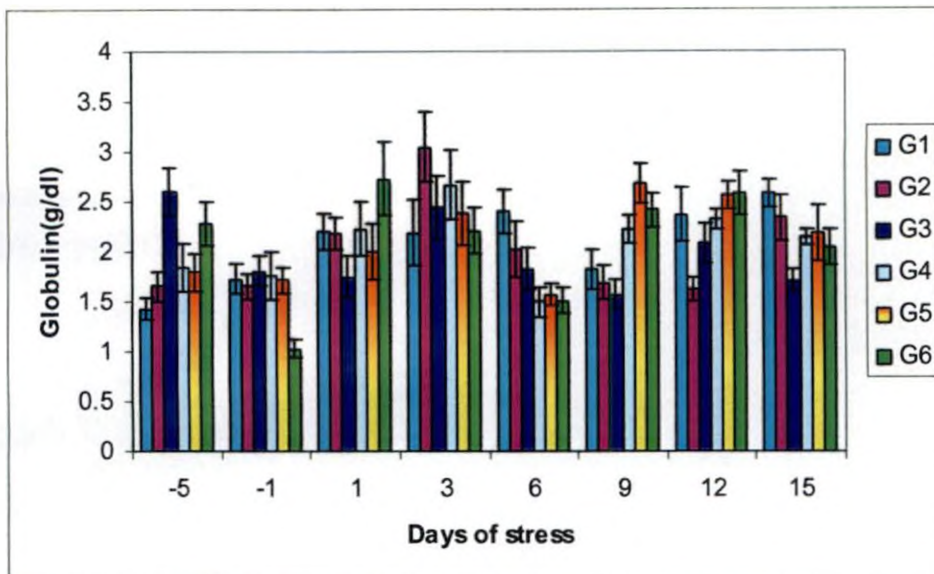
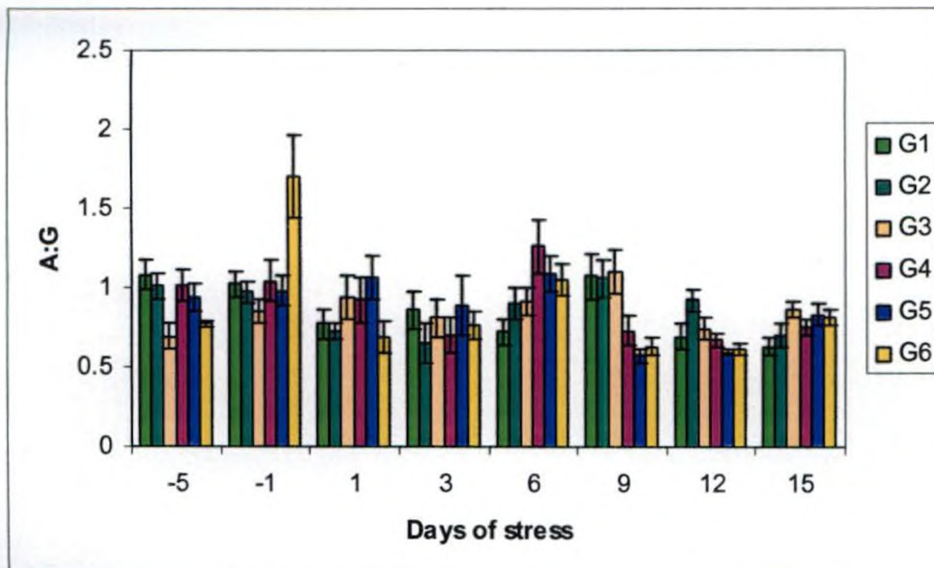


Figure 17. Albumin globulin ratio (A:G) of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

G5 chicken had a significant ($P<0.05$) decrease after nine (0.57 ± 0.04) and twelve (0.59 ± 0.02) days of stress and no significant difference one day before, after one day, three days, six days and fifteen days of stress.

G6 chicken had a significant ($P<0.05$) increase one day before (1.70 ± 0.26) and a decrease twelve days (0.61 ± 0.04) of stress and no significant difference after one day, six days, nine days and fifteen days of stress.

4.5.2 Serum Enzymes

4.5.2.1 Serum Alanine Amino Transferase (ALT)

The mean ± SE of ALT between all the experimental groups are given in Table 22 and Fig 18. Five days before stress G1 chicken (11.65 ± 0.82 U/l) had a significant ($P<0.05$) increase from G2 (7.57 ± 0.25 U/l) chicken and no significant difference from G3 chicken and G4 chicken. There was no significant difference between G2 and G3. G4 (10.77 ± 0.67 U/l) chicken had a significant increase ($P<0.05$) from G5 (8.29 ± 0.52 U/l) and G6 (8.36 ± 0.69 U/l) chicken. G5 chicken had no significant difference from G6 chicken.

One day before stress, G1 chicken had no significant difference from G2, G3 and G4 chicken. G2 (9.92 ± 0.55 U/l) chicken had a significant ($P<0.05$) increase value than G3 (7.55 ± 0.42 U/l) chicken and G4 (8.00 ± 0.35 U/l) chicken. G4 chicken had no significant difference from G5 and G6 chicken.

One day after stress there was no significant difference between G1 chicken, G2, G3 and G4 chicken. G4 chicken had no significant difference from G5 and G6 chicken.

At three day of stress G1 chicken (6.30 ± 0.28 U/l) had no significant difference from G2 and G4 chicken, but was significantly ($P\leq0.05$) lesser than G3 (11.42 ± 1.02 U/l) chicken. G4 (6.93 ± 0.14 U/l) chicken had a significantly ($P\leq0.05$) decreased value than G5 (11.43 ± 0.67 U/l) but was not significantly different from G6 chicken.

At six days of stress there was no significant difference among G1 chicken, G2, G3 and G4 chicken. G4 (7.51 ± 0.62 U/l) chicken had a significant (P<0.05) decrease value than G5 (14.08 ± 0.54 U/l) and G6 (11.18 ± 0.83 U/l) chicken. But G5 and G6 chicken had no significant difference between them.

Nine days after stress there was no significant difference between G1 chicken, G3 and G4 chicken. G2 (11.44±0.79) and G3 (7.84 ± 1.00 U/l) had a significant (P<0.05) increase than G1 (6.88 ± 0.43 U/l) and G4 (7.53 ± 0.94 U/l) chicken. G4 chicken had significant (P<0.05) decrease than G5 (11.30 ± 0.76 U/l) and G6 (12.48 ± 0.78 U/l) chicken. G5 chicken had no significant difference than G6 chicken.

After twelve days of stress G1 (10.88 ± 0.70 U/l) chicken had a significantly (P<0.05) increased value than G3 and no significant difference with G2 and G4. G2 (12.63 ± 0.85) chicken had a significant (P<0.05) increase than G3 (6.95 ± 0.51 U/l) and G4 (9.09 ± 0.73 U/l). G4 chicken had a significant (P<0.05) decrease than G5 (11.54 ± 0.51 U/l) and G6 (12.53 ± 0.45 U/l) chicken. G5 and G6 had no significant difference between them.

Fifteen days after stress G1 chicken (13.25 ± 0.28 U/l) had a significantly (P<0.05) increased value than G2 (10.96 ± 1.05 U/l) and G3 (9.33 ± 0.83 U/l) chicken. But there was no significant difference with G4 chicken. G4, G5 and G6 chicken had no significant difference among themselves.

The ALT between days were compared with values obtained five days before the induction of stress in each group. In G1 chicken ALT value had a significant (P<0.05) decrease one day before (8.97 ± 0.71 U/l), after one (8.35 ± 1.23 U/l), three (6.30 ± 0.28 U/l), six (7.72 ± 1.09 U/l) and nine days (6.88 ± 0.43 U/l) and an increase on the fifteenth day (13.25 ± 0.28 U/l) of stress. There was no significant difference on twelth day of stress.

G2 chicken had a significant (P<0.05) increase one day before (9.92 ± 0.55 U/l), one day (9.37 ± 0.78 U/l), nine days (11.44 ± 0.79 U/l), twelve days

(12.63 ± 0.85) and fifteen days (10.96 ± 1.05 U/l) of stress and no significant difference three days and six days of stress.

G3 chicken had a significant (P<0.05) decrease one day before (7.55 ± 0.42 U/l) and twelve days (6.95 ± 0.51 U/l) of stress and no significant difference on one day, three, six, nine and fifteen days of stress.

G4 chicken had a significant (P<0.05) decrease one day before (8.00 ± 0.35), one day (7.50 ± 0.45), three days (6.93 ± 0.14 U/l), six days (7.51 ± 0.62 U/l), nine days (7.53 ± 0.94 U/l) and a significant (P<0.05) increase on fifteenth day (12.63 ± 0.11 U/l) of stress. There was no significant difference on twelth day of stress.

G5 chicken had a significant (P<0.05) increase three days (11.43 ± 0.67 U/l), six days (14.08 ± 0.54 U/l), nine days (11.30 ± 0.76 U/l), twelve days (11.54 ± 0.51 U/l) and fifteen days (11.53 ± 0.71 U/l) of stress and no significant difference one day before and one day after stress.

G6 chicken had a significant (P<0.05) increase after six days (11.18 ± 0.83 U/l), nine days (12.48 ± 0.78 U/l) twelve days (12.53 ± 0.45 U/l) and fifteen days (12.34 ± 0.40 U/l) of stress and a significant (P<0.05) decrease one day after stress (6.37±0.23) and no significant difference one day before and three days after stress.

4.5.3 Serum Lipid Profile

4.5.3.1 Serum Total Cholesterol

The mean ± SE of total cholesterol between all the experimental groups are given in Table 23 and Fig 19. At five day before stress G1 (168.49 ± 11.49 mg/dl) chicken had a significant (P<0.05) increase in total cholesterol than G4 (133.70 ± 10.26 mg/dl) chicken and a significant (P<0.05) decrease than G3 (179.70 ± 8.76 mg/dl). There was no significant difference was noticed between G1 and G2 chicken. There was no significant difference between G2 and G3

chicken. G4 chicken had a significantly ($P<0.05$) decreased value than G5 (174.10 ± 13.12 mg/dl) and G6 (176.30 ± 8.04 mg/dl) chicken. There was no significant difference between G5 and G6 chicken.

After nine days of stress no significant difference was observed among G1, G2 and G3 chicken. G4 (158.10 ± 4.70 mg/dl) chicken had a significant ($P<0.05$) increase than G1 (134.60 ± 9.72 mg/dl) chicken but there was no significant difference between G2 and G3 chicken. G4 chicken had a significantly ($P<0.05$) increased value than G5 (138.90 ± 7.06 mg/dl), and G6 (134.50 ± 6.54 mg/dl) chicken had a significantly ($P<0.05$) lower value than G5 chicken.

There was no significant difference among G1, G2 and G3 chicken after twelve days of stress. G1 (135.60 ± 6.47 mg/dl) chicken had a significant ($P<0.05$) decrease from G4 (162.90 ± 4.67 mg/dl) chicken. G4 chicken had a significantly ($P<0.05$) increased value from G5 (142.20 ± 8.31 mg/dl) and G6 (140.40 ± 4.51 mg/dl) chicken. There was no significant difference between G5 and G6 chicken.

After fifteen days of stress there was no significant difference among G1, G2 and G3 chicken but G4 (162.50 ± 5.47 mg/dl) chicken had a significant ($P<0.05$) increase than G2 (118.00 ± 14.63 mg/dl) and G3 (136.30 ± 8.10 mg/dl). G4 chicken had a significant ($P<0.05$) increase than G5 (132.00 ± 7.90 mg/dl) and G6 (136.30 ± 6.58 mg/dl). G5 and G6 chicken had no significant difference among them.

Serum cholesterol concentrations between days within groups were compared with values obtained five days before the induction of stress in each group. G1 chicken had a significant ($P<0.05$) decrease one day before (157.50 ± 9.93 mg/dl), after one day (139.90 ± 11.59 mg/dl), three days (143.20 ± 10.52 mg/dl), six days (142.80 ± 11.19 mg/dl), nine days (134.60 ± 9.72 mg/dl) and twelve days (135.60 ± 6.47 mg/dl) of stress and no significant difference on fifteenth day of stress.

Table 22. Serum alanine amino traferase (ALT) of experimental groups (Mean ± SE, n=10)

ALT (U/l)								
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	11.05^{b}±0.82	8.97^{ab*}±0.71	8.35^{ab*}±1.23	6.30^{a*}±0.28	7.72^{a*}±1.09	6.88^{a*}±0.43	10.88^{bc}±0.70	13.25^{c*}±0.28
G 2	7.57^{a}±0.25	9.92^{b*}±0.55	9.37^{b*}±0.78	7.47^{ab}±0.56	7.14^{a}±0.56	11.44^{b*}±0.79	12.63^{c*}±0.85	10.96^{ab*}±1.05
G 3	9.39^{ab}±1.00	7.55^{a*}±0.42	10.25^{b}±1.41	11.42^{c}±1.02	8.97^{ab}±1.22	7.84^{a}±1.00	6.95^{a*}±0.51	9.33^{a}±0.83
G 4	10.77^{b}±0.67	8.00^{a*}±0.35	7.50^{ab*}±0.45	6.93^{ab*}±0.14	7.51^{a*}±0.62	7.53^{a*}±0.94	9.09^{b}±0.73	12.63^{bc*}±0.11
G 5	8.29^{a}±0.52	8.30^{a}±0.57	9.80^{b}±1.08	11.43^{c*}±0.67	14.08^{c*}±0.54	11.30^{b*}±0.76	11.54^{c*}±0.51	11.53^{bc*}±0.71
G 6	8.36^{a}±0.69	7.97^{a}±0.38	6.37^{a*}±0.23	8.30^{b}±0.59	11.18^{b*}±0.83	12.48^{b*}±0.78	12.53^{c*}±0.45	12.34^{bc*}±0.40

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.
Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.
In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.
*denotes significant difference (P<0.05) within a row from five days before stress.

Table 23. Serum cholesterol of experimental groups (Mean ± SE, n=10)

	Total cholesterol (mg/dl)							
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	$168.49^{b}\pm11.49$	$157.50^{*}\pm9.93$	$139.90^{*}\pm11.59$	$143.20^{*}\pm10.52$	$142.80^{*}\pm11.19$	$134.60^{a*}\pm9.72$	$135.60^{a*}\pm6.47$	$133.20^{a}\pm5.122$
G 2	$150.30^{bc}\pm10.64$	150.80 ± 12.03	134.30 ± 13.78	142.50 ± 9.13	143.80 ± 7.76	$124.70^{a*}\pm5.88$	$133.80^{a}\pm5.12$	$118.00^{a*}\pm4.63$
G 3	$179.70^{c}\pm8.76$	165.70 ± 9.62	158.30 ± 10.82	$142.80^{*}\pm8.99$	$143.50^{*}\pm9.11$	$137.90^{ab*}\pm7.70$	$139.30^{a*}\pm6.75$	$136.30^{a*}\pm8.10$
G 4	$133.70^{a}\pm10.26$	$129.20^{*}\pm9.91$	133.10 ± 10.07	142.60 ± 8.11	152.50 ± 5.75	$158.10^{b*}\pm4.70$	$162.90^{b*}\pm4.67$	$162.50^{b*}\pm5.47$
G 5	$174.10^{b}\pm13.12$	$157.90^{*}\pm9.43$	$140.60^{*}\pm9.61$	$129.30^{*}\pm4.68$	$142.80^{*}\pm8.72$	$138.90^{c*}\pm7.06$	$142.20^{a*}\pm8.31$	$132.00^{a*}\pm7.90$
G 6	$176.30^{b}\pm8.04$	$159.30^{*}\pm8.00$	$152.20^{*}\pm6.90$	$139.90^{*}\pm7.87$	$144.30^{*}\pm3.11$	$134.50^{a*}\pm6.54$	$140.40^{a*}\pm4.51$	$136.30^{a*}\pm6.58$

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.
Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.
In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.
* denotes significant difference (P<0.05) within a row from five days before stress.

Figure 18. Serum alanine amino transferase (ALT) of experimental groups (Mean ± SE, n=10)

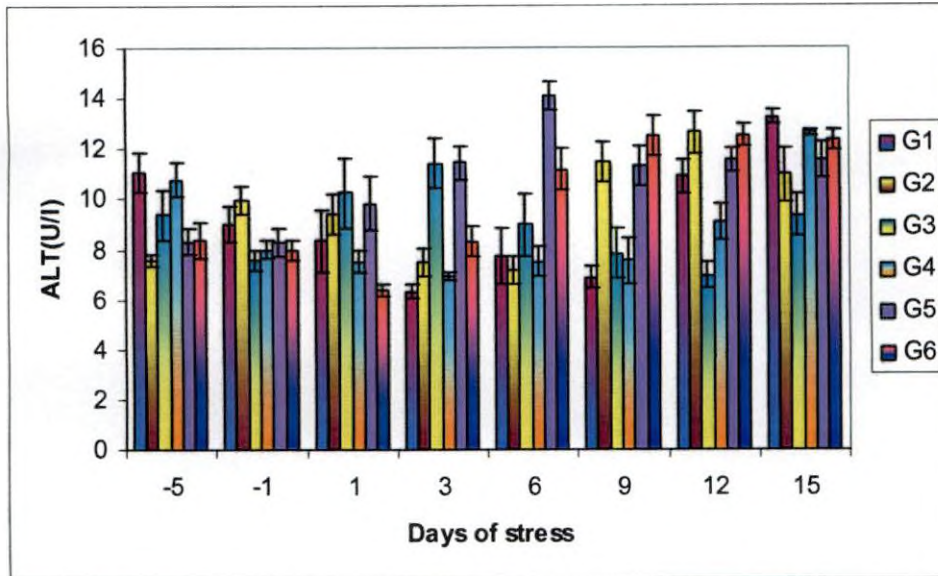
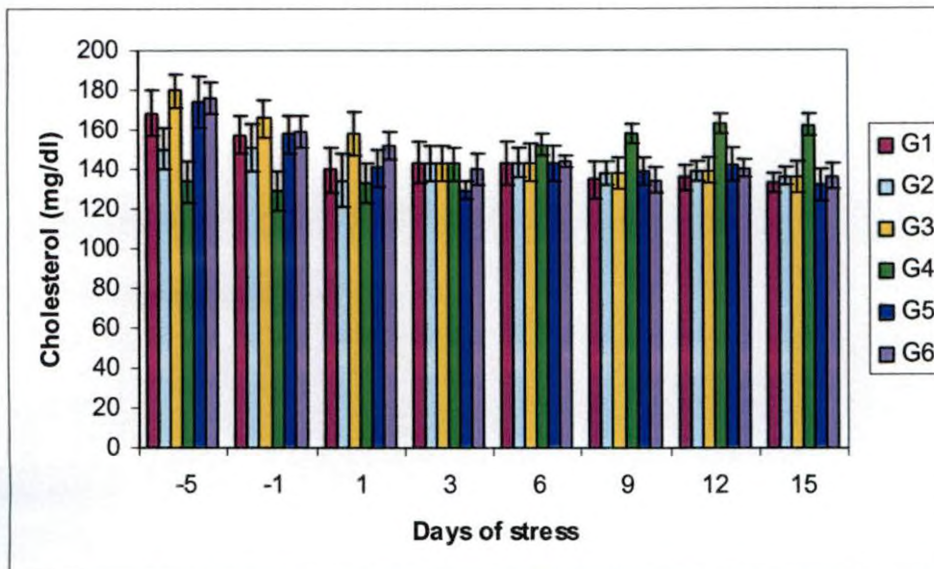


Figure 19. Serum cholesterol of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

G2 chicken had a significant (P<0.05) decrease after nine (124.70 ± 5.88 mg/dl) and fifteen days (118.00 ± 4.63 mg/dl) of stress and no significant difference one day before, one, three, six and twelve days of stress.

G3 chicken had a significant (P<0.05) decrease three days (142.80 ± 8.99 mg/dl), six days (143.50 ± 9.11 mg/dl), nine days (137.90 ± 7.70 mg/dl), twelve days (139.30 ± 6.75 mg/dl) and fifteen days (136.30 ± 8.10 mg/dl) of stress and no significant difference one day before and after one day of stress.

G4 chicken had a significant (P<0.05) decrease one day before (129.20 ± 9.91 mg/dl) and a significant (P<0.05) increase after nine days (158.10 ± 4.70 mg/dl) twelve days (162.90 ± 4.67 mg/dl) and fifteen days (162.50 ± 5.47 mg/dl) of stress. There was no significant difference after one day, three days and six days of stress.

G5 chicken had a significant (P<0.05) decrease one day before (157.90 ± 9.43 mg/dl), after one day (140.60 ± 9.61 mg/dl), three days (129.30 ± 4.68 mg/dl), six days (142.80 ± 8.72 mg/dl) and nine days (138.90 ± 7.06 mg/dl) twelve days (14.20 ±8.31) and fifteen days (132.00 ± 7.90 mg/dl) of stress.

G6 chicken had a significant (P<0.05) decrease one day before (159.30 ± 8.00), one day (152.20 ± 6.90 mg/dl), three days (139.90 ± 7.87 mg/dl) six days (144.30 ± 3.11 mg/dl), nine days (134.50 ± 6.54 mg/dl), twelve days (140.40 ± 4.51 mg/dl) and fifteen days (136.30 ± 6.58 mg/dl) of stress.

4.5.4 Serum Glucose

The mean ± SE of serum glucose between all the experimental groups are given in Table 24 and Fig 20. Five days before stress there was no significant difference on serum glucose among G1, G2, G3 and G4 chicken. G5 (185.90 ± 6.33 mg/dl) and G6 (186.80 ± 9.84 mg/dl) chicken had a significant (P<0.05) increase than G4 (158.50 ± 11.85 mg/dl). There was no significant difference between G5 and G6 chicken.

After three day of stress there was a significant ($P<0.05$) increase in serum glucose was observed in G4 (190.50 ± 9.83 mg/dl) compared to G1 (155.60 ± 9.35 mg/dl), G2 (142.10 ± 6.72 mg/dl) and G3 (150.70 ± 9.53 mg/dl) chicken. There was no significant difference was observed between G1, G2 and G3 chicken. No significant difference was observed among G4, G5 and G6 chicken.

After twelve days of stress a significant ($P<0.05$) increase value was observed in G4 (207.60 ± 11.76 mg/dl) chicken than G1 (155.90 ± 7.79 mg/dl), G2 (147.20 ± 6.29 mg/dl) and G3 (153.60 ± 10.68 mg/dl) chicken. No significant difference was seen between G4 and G5 chicken. There was a significant ($P<0.05$) increase in G4 chicken than G6 chicken (165.40 ± 10.71 mg/dl).

Serum glucose between days within groups were compared with values obtained five days before the induction of stress in each group. G1 chicken had no significant difference between days of stress.

G2 chicken had a significant ($P<0.05$) decrease three days (142.10 ± 6.72 mg/dl), and twelve days (147.20 ± 6.29 mg/dl) after stress and no significant difference one day before, after one day, six days, nine days and fifteen days of stress.

G3 chicken had a significant ($P<0.05$) decrease after three days (150.70 ± 9.53 mg/dl) and twelve days (153.60 ± 10.68 mg/dl) of stress and no significant difference one day before, one day, six days, nine days and fifteen days of stress.

G4 chicken had a significant ($P<0.05$) increase after three days (190.50 ± 9.83 mg/dl), twelve days (207.60 ± 11.76 mg/dl) and fifteen days (180.90 ± 9.28 mg/dl) of stress and no significant difference one day before, one day, six days, and nine days of stress.

G5 chicken had a significant ($P<0.05$) decrease one day before (165.30 ± 6.30 mg/dl), after one day (165.00 ± 10.10 mg/dl) six days (168.00 ± 6.87 mg/dl) and fifteen days (162.10 ± 7.85 mg/dl) of stress and no significant difference three

days, nine days, twelve days after stress. G6 chicken had a significant ($P<0.05$) decrease on one day before (154.70 ± 6.94 mg/dl) and fifteen days (168.80 ± 5.25 mg/dl) of stress and no significant difference one day, three days, six days, nine days and twelve days of stress.

4.5.5 Serum C - reactive protein (CRP) level

The serum C-reactive protein are presented in Table 25 and Fig 21. The value of C-reactive protein on ninth day of stress on G1, G4, G5 and G6 chicken were 0.24 mg/dl, 1.92 mg/dl, 0.96 mg/dl, and 0.48 mg/dl respectively after nine days of stress. The value of stressed chicken (G4) was 1.92 mg/dl and it was lesser in G5 and G6 chicken. Chicken supplemented with *W. somnifera* (G5 and G6) had a decreased C reactive protein level than stressed chicken (G4).

Table 24. Serum glucose of experimental groups (Mean ± SE, n=10)

Serum glucose (mg/dl)								
Days / Groups	-5	-1	1	3	6	9	12	15
G 1	154.70^{a} ±9.95	153.90±5.59	151.60±3.57	155.60^{ab} ±9.35	178.60±7.80	150.70±7.80	155.90^{ab} ±7.79	170.30±11.74
G 2	170.40^{ab}±6.46	172.50±8.48	171.10±6.23	142.10$^{a\,*}$±6.72	157.80±5.41	167.20±6.84	147.20$^{a\,*}$±6.29	161.00±2.79
G 3	170.70^{ab}±6.36	163.90±8.53	163.70±11.7	150.70$^{a\,*}$±9.53	179.90±9.45	155.30±8.85	153.60$^{ab\,*}$±10.68	166.80±4.26
G 4	158.50^{a} ±11.85	165.10±7.11	156.50±4.23	190.50$^{c\,*}$±9.83	166.90±6.08	163.40±6.88	207.60$^{c\,*}$±11.76	180.90*±9.28
G 5	185.90^{b} ±6.33	165.30*±6.30	165.00*±10.1	179.80^{bc} ±7.06	168.00*±6.87	170.90±10.22	183.10^{bc} ±13.25	162.10*±7.85
G 6	186.80^{b} ±9.84	154.70*±6.94	178.80±9.23	178.30^{bc}±7.10	169.10±6.42	164.90±10.20	165.40^{ab} ±10.71	168.80*±5.25

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

* denotes significant difference (P<0.05) within a row from five days before stress.

Table 25. C reactive protein of experimental groups (Mean , n=10)

C-reactive protein (mg/dl)	
Days / Groups	9 days of stress
G1	0.24
G4	1.92
G5	0.96
G6	0.48

G1- Normal , G4- Stressed, G5- Stressed with 5 g *W. somnifera* / kg feed, and G6- Stressed with 10 g *W. somnifera* / kg feed.

Figure 20. Serum glucose of experimental groups (Mean ± SE, n=10)

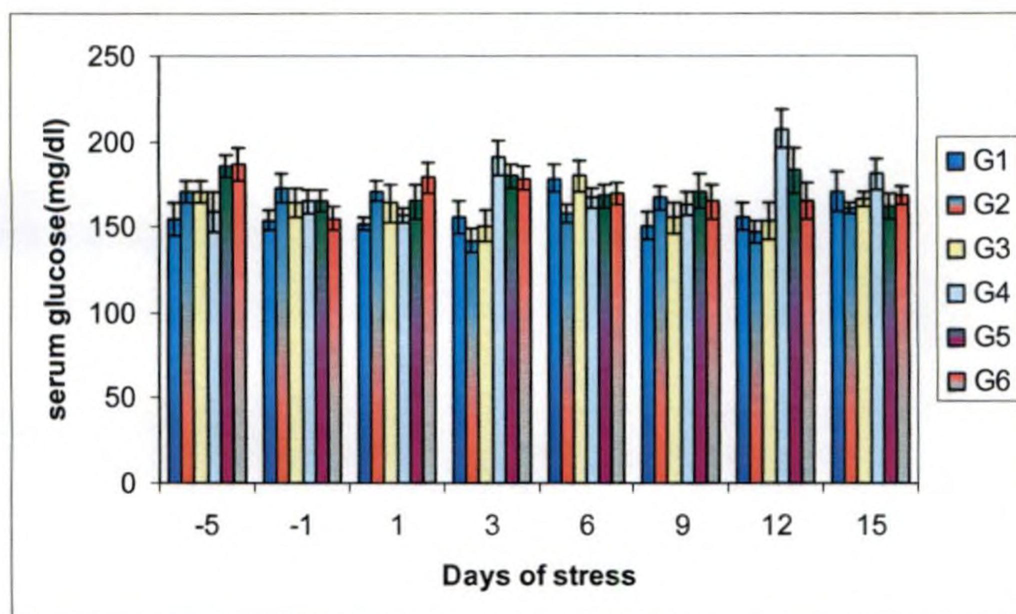
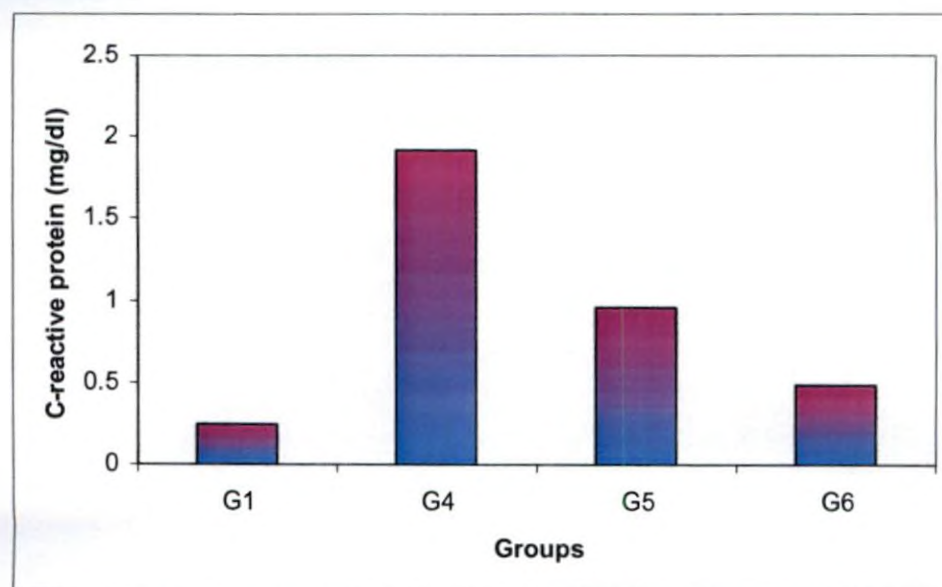


Figure 21. Serum C- reactive protein of experimental groups (Mean, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

4.6 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON LIVER LIPID PEROXIDATION AND REDUCED GLUTATHIONE OF BROILER CHICKEN

4.6.1 Lipid Peroxidation

The lipid peroxidation (mean ± SE) between all the experimental groups are presented in the Table 26 and Fig 22 a. The value of lipid peroxidation in G1, G2, G3, G4, G5 and G6 birds were 263.20±33.06, 195.00±34.06, 152.60±9.60, 668.10±44.51, 530.30 ±35.78 and 442.90 ±27.79 nmol / g of wet tissue respectively. The G1 (263.20±33.06 nmol /g of wet tissue) chicken had a significantly ($P<0.05$) increased value compared to G3 (152.60±9.60 nmol /g of wet tissue) chicken. G4 chicken had significantly ($P<0.05$) increased than G1, G2 (195.00±34.06 nmol /g of wet tissue) and G3 chicken. G2 chicken had no significant difference with G3 chicken. G4 (668.10±44.51 nmol /g of wet tissue) chicken had a significant ($P<0.05$) increase than G5 (530.30 ±35.78 nmol /g of wet tissue) and G6 (442.90 ±27.79 nmol /g of wet tissue) chicken. G5 chicken had no significant difference with G6 chicken.

4.6.2 Reduced Glutathione

The reduced glutathione (mean ± SE) between all the experimental groups are presented in the Table 26 and Fig 22 b. The value (mean ± SE) of liver reduced glutathione in G1, G2,G3,G4, G5 and G6 chicken were 5100±158.46, 6620±246.67, 7080±143.60, 4180±118.13, 6750 ±240.00 and 6840±180.86 µg/g of wet tissue. G4 chicken had a significantly ($P<0.05$) lower level than G1 (5100±158.46 µg/g of wet tissue), G2 (6620±246.67 µg/g of wet tissue) and G3 (7080±143.60 µg/g of wet tissue) chicken. G2 chicken had no significant difference with G3 chicken groups. G4 (4180±118.13 µg/g of wet tissue) chicken

Table 26. Liver tissue lipid peroxidation (LP) and reduced glutathione (GSH) level of experimental groups (Mean ± SE, n=10)

Parameters / Groups	LP nmol /g wet tissue.	GSH µg/g wet tissue
G 1	263.20^{b} ±33.06	5100.00^{b} ±158.46
G 2	195.00^{ab} ±34.06	6620.00^{c} ±246.67
G 3	152.60^{a} ±9.60	7080.00^{c} ±143.60
G 4	668.10^{d} ±44.51	4180.00^{a} ±118.13
G 5	530.30^{c} ±35.78	6760.00^{c} ±240.00
G 6	442.90^{c} ±27.79	6840.00^{c} ±180.86

G1- Normal , G2- Normal with 5 g *W. somnifera* / kg feed, G3- Normal with 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed with 5 g *W. somnifera* / kg feed, and G6- Stressed with 10 g *W. somnifera* / kg feed.
Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.
In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

Figure 22 a. Lipid peroxidation level of experimental groups (Mean ± SE, n=10)

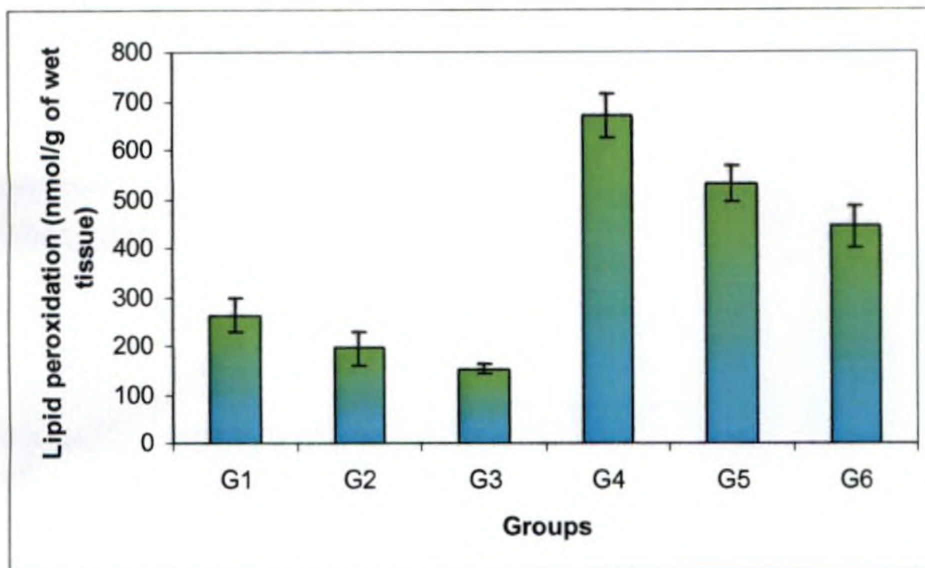
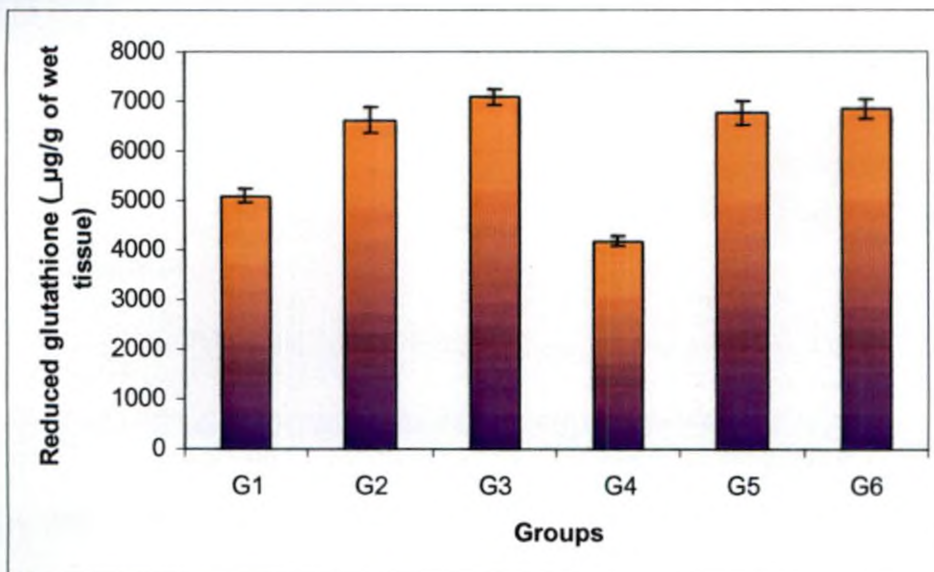


Figure 22 b. Reduced glutathione level of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

had a significantly ($P<0.05$) decreased value than G5 (6750±240.00 µg/g of wet tissue) chicken and G6 chicken. G5 chicken had no significant difference with G6 (6840±180.86 µg/g of wet tissue) chicken.

4.7 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON IMMUNOLOGICAL PARAMETER OF BROILER CHICKEN

The mean ± SE of HA titres between all the experimental groups are given in Table 27 and Fig 23. One day after stress G1 chicken had a significantly ($P<0.05$) reduced level than rest of the groups. There was no significant difference among G2, G3, G4, G5 and G6 chicken.

After twelve days of stress G1 chicken did not show any significant difference with G2, G3 and G4 chicken. There was no significant difference between G2 and G3 chicken. G4 chicken did not express any significant difference with G5 and G6 chicken. G5 and G6 chicken did not indicate any significant difference between them.

HA titre between days was compared with one day before stress. G1 chicken had a significant ($P<0.05$) decrease after six days (3.00 ± 0.45), nine days (1.90 ± 0.28), twelve days (1.00 ± 0.21) and fifteen days (0.20 ± 0.20) of stress and no significant difference after one day and three days of stress.

G2 chicken had a significant ($P<0.05$) increase one day (5.90 ± 0.41) after stress and a significant ($P<0.05$) decrease six days (4.20 ± 0.51), nine days (2.30 ± 0.33), twelve days (1.60 ± 0.43) and fifteen days (.090 ± 0.35) of stress and no significant difference after three days of stress.

G3 chicken had a significant ($P<0.05$) increase one day (6.90 ± 0.69) and a significant ($P<0.05$) decrease after six days (4.10 ± 0.50), nine days (2.80 ± 0.55), twelve days (1.80 ± 0.47) and fifteen days (0.60 ± 0.31) of stress. There was no significant difference on third day of stress.

G4 chicken had a significant ($P<0.05$) increase after one day (6.40 ± 0.56) stress and a significant ($P<0.05$) decrease after nine days (1.80 ± 0.44), twelve days (0.60 ± 0.16) and fifteen days (0.20 ± 0.13) of stress and no significant difference after three and six days of stress.

G5 chicken had a significant ($P<0.05$) increase after one day (6.00 ± 0.49) of stress and a significant ($P<0.05$) decrease after six days (3.30 ± 0.42), nine days (2.00 ± 0.33), twelve days (0.80 ± 0.20) and fifteen days (0.40 ± 0.16) of stress and no significant difference after three day of stress.

G6 chicken had a significant ($P<0.05$) increase after one day (6.40 ± 0.43) and a significant ($P<0.05$) decrease after nine day (3.20 ± 0.68), twelve days (0.80 ± 0.20) and fifteen days (0.40 ± 0.16) of stress. There was no significant difference after three days and six days of stress.

4.8 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON GASTROINTESTINAL ENZYMES OF BROILER CHICKEN

4.8.1 Proventricular Protease

The protease activity (mean ± SE) between all the experimental groups are presented in Table 28 and Fig 24 a. The protease activity in G1, G2, G3, G4, G5 and G6 chicken were 237.20 ± 20.41, 289.80 ± 20.21, 316.99 ± 50.09, 164.00 ± 10.20, 187.70 ± 26.16 and 243.20 ± 26.47 Pepsin unit/g of tissue respectively. There was no significant difference among G2 chicken, G3 chicken and G4 chicken. There was no significant difference between G4, G5 and G6 chicken.

4.8.2 Intestinal Amylase

The amylase activities (mean ± SE) between all the experimental groups are presented in Table 28 and Fig 24 b. The amylase activity in G1, G2, G3, G4,

G5 and G6 chicken were 17.03 ± 0.40, 20.51 ± 0.99, 20.27 ± 1.03, 15.06 ± 1.28, 16.17 ± 0.93 and 18.94 ± 0.50 Somogyi units/g of tissue respectively. The G1 (17.03 ± 0.40 Somogyi units/g of tissue) chicken had a significantly (P<0.05) decreased value than G2 (20.51 ± 0.99 Somogyi units/g of tissue) chicken, G3 chicken and no significant difference with G4 chicken. G2 chicken did not have a significant difference with G3 chicken. G4 (15.06 ± 1.28 Somogyi units/g of tissue) chicken had no significant difference with G5 chicken but had a significantly (P<0.05) decreased value than G6 (18.94 ± 0.50 Somogyi units/g of tissue) chicken. G5 (16.17 ± 0.93 Somogyi units/g of tissue) chicken had a significant (P<0.05) decrease than G6chicken.

4.8.3 Pancreatic Lipase

The lipase activity (mean ± SE) between all the experimental groups are presented in Table 28 and Fig 24 c. The lipase activity in G1, G2, G3, G4, G5 and G6 chicken were 23.17 ± 0.57, 24.56 ± 0.86, 26.33±0.39, 15.75 ± 0.64, 21.62 ± 0.46 and 24.28 ± 0.42 lipase units/g of tissue respectively. The G1 (23.17 ± 0.57 lipase units/g of tissue) chicken had a significant (P<0.05) decrease than G3 (26.33±0.39 lipase units/g of tissue) and G4 chicken and no significant difference with G2 chicken. G2 (24.56 ± 0.86 lipase units/g of tissue) chicken had a significantly (P<0.05) decreased value than G3 chicken. G4 (15.75 ± 0.64 lipase units/g of tissue) chicken had a significantly (P<0.05) decreased value than G5 (21.62 ± 0.46 lipase units/g of tissue) and G6 (24.28 ± 0.42 lipase units/g of tissue) chicken. G5 chicken had a significantly (P<0.05) decreased level than G6 chicken.

Table 27. Antibody titre of experimental groups (Mean ± SE, n=10)

Groups \ Days	Haemagglutinin titre ($\log_2$)						
	-1	1	3	6	9	12	15
G 1	3.90±0.41	$4.10^{a}\pm0.31$	4.70±0.62	$3.00^{*}\pm0.45$	$1.90^{*}\pm0.28$	$1.00^{ab*}\pm0.21$	$0.21^{*}\pm0.20$
G 2	5.10±0.46	$5.90^{b*}\pm0.41$	4.80±0.33	$4.20^{*}\pm0.51$	$2.30^{*}\pm0.33$	$1.60^{abc*}\pm0.43$	$0.90^{*}\pm0.35$
G 3	5.80±0.66	$6.90^{b*}\pm0.69$	5.90±0.38	$4.10^{*}\pm0.50$	$2.80^{*}\pm0.55$	$1.80^{bc*}\pm0.47$	$0.60^{*}\pm0.31$
G 4	5.60±0.58	$6.40^{b*}\pm0.56$	5.20±0.36	3.70±0.60	$1.80^{*}\pm0.44$	$0.60^{a*}\pm0.16$	$0.20^{*}\pm0.13$
G 5	4.80±0.39	$6.00^{b*}\pm0.49$	5.10±0.35	$3.30^{*}\pm0.42$	$2.00^{*}\pm0.33$	$0.80^{ab*}\pm0.20$	$0.40^{*}\pm0.16$
G 6	5.50±0.34	$6.40^{b*}\pm0.43$	4.90±0.46	4.30±0.67	$3.20^{*}\pm0.68$	$0.80^{ab*}\pm0.20$	$0.40^{*}\pm0.16$

-5 and -1 – days before stress and 1, 3, 6, 9, 12, and 15- days after stress. G1- Normal , G2- Normal with 5 g *W. somnifera* / kg feed, G3- Normal with 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed with 5 g *W. somnifera* / kg feed, and G6- Stressed with 10 g *W. somnifera* / kg feed.

Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.

In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

*denotes significant difference (P<0.05) within a row from one day before stress.

Table 28. Gastrointestinal enzymes of experimental groups (Mean ± SE, n=10)

	Gastrointestinal enzymes		
Groups	Protease (Pepsin unit/ g of tissue)	Amylase (Somogyi units / g of tissue)	Lipase (Lipase units / g of tissue)
G 1	$237.20^{ab} \pm 20.41$	$17.03^{ab} \pm 0.40$	$23.17^{bc} \pm 0.57$
G 2	$289.80^{b} \pm 20.21$	$20.51^{c} \pm 0.99$	$24.56^{c} \pm 0.86$
G 3	$316.90^{b} \pm 50.09$	$20.27^{c} \pm 1.03$	$26.33^{d} \pm 0.39$
G 4	$164.00^{ab} \pm 10.20$	$15.06^{a} \pm 1.28$	$15.75^{a} \pm 0.64$
G 5	$187.70^{a} \pm 26.16$	$16.17^{a} \pm 0.93$	$21.62^{b} \pm 0.46$
G 6	$243.20^{ab} \pm 26.47$	$18.94^{bc} \pm 0.50$	$24.28^{c} \pm 0.42$

G1- Normal , G2- Normal with 5 g *W. somnifera* / kg feed, G3- Normal with 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed with 5 g *W. somnifera* / kg feed, and G6- Stressed with 10 g *W. somnifera* / kg feed.
Means bearing the same superscript in the column do not differ significantly (P>0.05) between groups.
In a column if there is no superscript it means no significant difference (P>0.05) among six treatments.

Figure 23. Antibody titre of experimental groups (Mean ± SE, n=10)

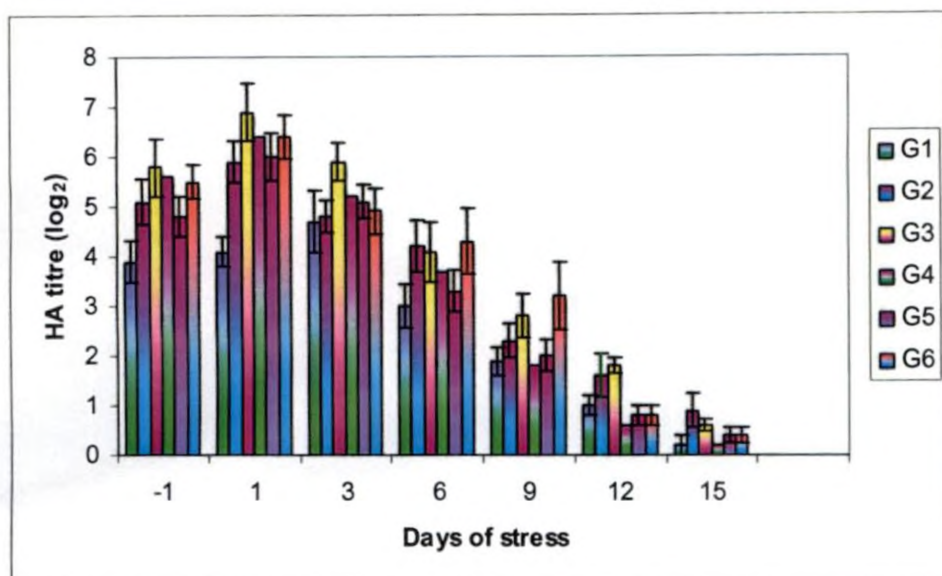
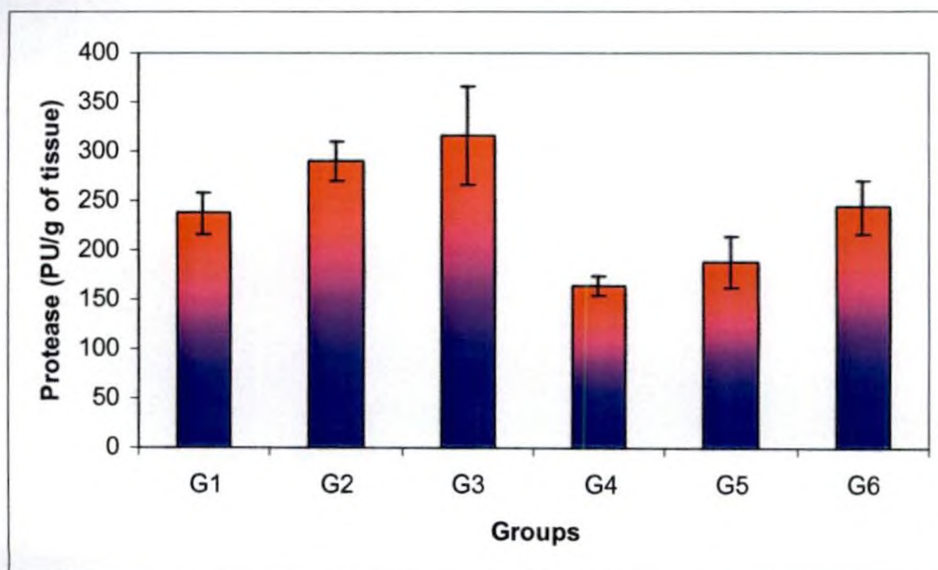


Figure 24 a. Proventricular protease of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Figure 24 b. Intestinal amylase of experimental groups (Mean ± SE, n=10)

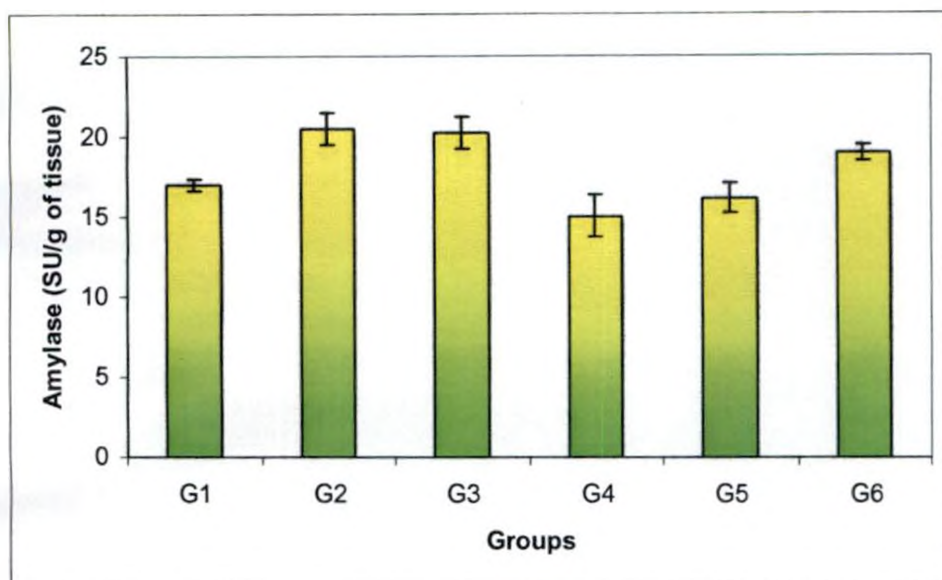
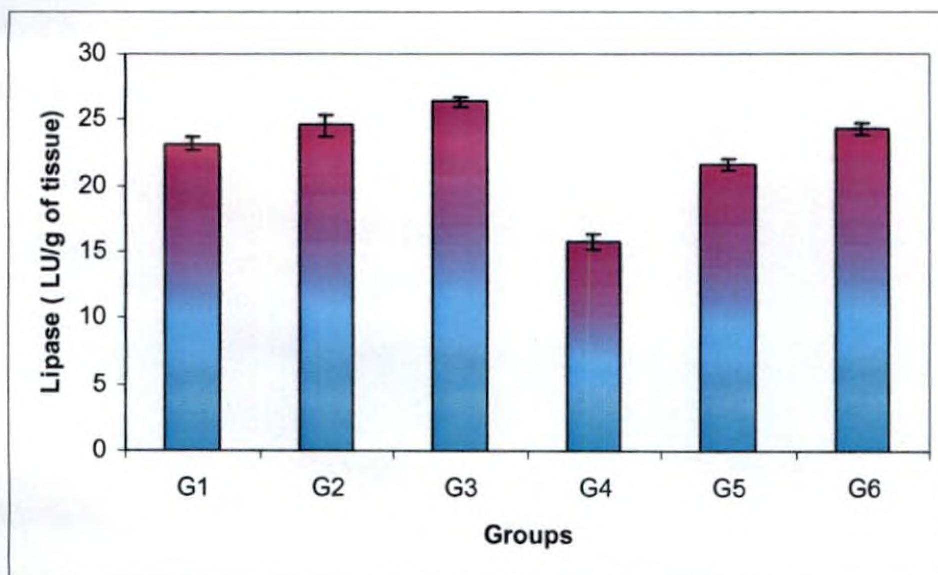


Figure 24 c. Pancreatic lipase of experimental groups (Mean ± SE, n=10)



G1- Normal , G2- Normal + 5 g *W. somnifera* / kg feed, G3- Normal + 10 g *W. somnifera* / kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* / kg feed, and G6- Stressed + 10 g *W. somnifera* / kg feed.

Discussion

5. DISCUSSION

5.1 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON BODY WEIGHT OF BROILER CHICKEN

5.1.1 Body Weight

Body weight is considered as an index of the nutritional status of animal (Bhosale and Rao, 2001). Overall result of the study indicated that the weekly body weight of *Withania somnifera* supplemented broilers were similar to that of control birds. At the end of the study (six weeks of age), there was no significant difference between the body weight of normal birds and birds supplemented with 5 g *W. somnifera* / kg of feed and 10 g *W. somnifera* / kg of feed. This finding is in agreement with the results of Samarth *et al.* (2002) where supplementation of 0.5 per cent *W. somnifera* / kg of feed mix in broiler chicken resulted in similar live body weight as that of non supplemented groups. However, Pedulwar *et al.* (2007) and Jadhav *et al.* (2008) reported an increase in body weight in *W. somnifera* supplemented groups and this could be attributed to the difference in strains used for the study.

The birds subjected to stress also indicated an increase in body weight with increase in age, but there was no significant increase in body weight during the last two weeks (fifth and sixth week of age). The stressed birds supplemented with *W. somnifera* exhibited an increase in body weight with increase in age but there was significant increase till end of the study. Though all the stressed birds had similar body weights, those birds not supplemented with *W. somnifera* had the least body weight. Reddy (2003) also did not observe any significant increase in body weight in Zeetress® supplemented stressed broiler chicken. The reduced body weight in stressed birds is similar to the findings of the Bolton *et al.*(1972), Feddes *et al.*(2002), Shivakumar *et al.*(2004), Dozier *et al.*(2005) and Samale *et al.* (2008), where stress due to overcrowding lowered the body weight of broiler

chicken. Among the stressed birds, those supplemented with *W. somnifera* had higher body weight and this was in agreement to the findings of Grandhi *et al.* (1994), where *W. somnifera* treated stressed mice showed increased body weight. It could be concluded that *W. somnifera* supplementation (5 g and 10 g *W. somnifera* / kg of feed) in broiler chicken diet did not affect the normal growth but has an effect in reducing stress related lowering of body weight.

5.1.2 Weekly Weight gain

The weekly weight gain improved for the birds from fourth week onwards till the end of the study. The highest gain was observed in normal birds supplemented with 10 g *W. somnifera* / kg of feed but statistically all the non stressed groups had similar weight gain at the end of the study. Mishra and Singh (2000) reported a non significant weight gain in broiler chicken supplemented with 0.5 per cent, 1 per cent and 2 per cent of *W. somnifera* in the diet from first to sixth week of age. Pedular *et al.* (2007), Jadhav *et al.* (2008) and Shisodiya *et al.* (2009) also reported an increase in body weight gain in broiler chicks supplemented with *W. somnifera.* In the present study also there was a significant increase in weekly weight gain in *W. somnifera* supplemented group.

Bhagarva *et al.* (1975), Pesti and Howrath (1983), Cravner *et al.* (1992), Shivakumar *et al.*(2004), Dozier *et al.* (2005) and Onbasilar and Aksoy (2008) reported that overcrowding stress reduced the body weight gain. In the present study there was insignificant reduction in body weight gain in stressed birds compared to non stressed birds, but the stressed birds supplemented with *W. somnifera* expressed a higher body weight gain than non supplemented stressed birds. Wheeler (1994) reported that *W. somnifera* reduced the effect of stress in intensively housed chicken. Similar antistress property of *W. somnifera* was also observed by Kaur *et al.* (2001) and Bhattacharya and Murugandam (2003) in stressed birds. It could be inferred that the stress ameliorating effect of *W. somnifera,* might have contributed to the non significant increase in body weight gain to *W. somnifera* supplemented group of birds.

5.2 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON FEED CONSUMPTION AND FEED EFFICIENCY OF BROILER CHICKEN

5.2.1 Weekly Feed Consumption

The birds supplemented with *W. somnifera* expressed an increase in feed consumption than non supplemented groups. Aphale (1998) reported an increase in feed consumption in rats supplemented with a combination of Ginseng and *W. somnifera.* Similar increase in feed consumption was also observed by Samarth *et al.* (2002) in *W. somnifera* supplemented broiler chicken. Akotkar *et al.* (2007) found an increase in feed consumption for broilers supplemented with 0.5 per cent to 1 per cent *W. somnifera* / kg of feed in diet, which was in agreement with the present study.

Tomgave and Seeger (1945) reported that reduction in floor space lowered feed consumption in broiler chicken. Kuan *et al.* (1990) also found out that increasing stocking density (stress) reduced the feed consumption in broiler chicken. Similar results were also reported by Shivakumar *et al.* (2004), Dozier *et al.* (2005) and Samale *et al.* (2008) in broiler chicken subjected to overcrowding stress. In the present study also the feed consumption was lesser in stressed birds. The study revealed a higher feed consumption in stressed birds supplemented with *W. somnifera* than stressed birds. The feed consumption was higher in 10 g *W. somnifera* / kg of feed than 5 g *W. somnifera* / kg of feed supplemented group. Wheeler (1994) reported that use of *W. somnifera* reduced the effect of stress in intensively housed chicken and improved feed consumption. Rajeshwari *et al.* (2001) reported that Zeetress® supplemented broiler chickens had an improved feed intake. Narayananswamy *and* Santhosh Kumar (2004) reported that Geriforte® reduced summer stress and improved feed consumption in broiler chicken. These findings all are in agreement with the results of the present study. It could be concluded that *W. somnifera* supplementation could reduce stress induced by stocking density and improved the feed consumption.

5.2.2 Weekly Feed Efficiency

The feed efficiency at six weeks of age revealed that the efficiency was better in *W. somnifera* supplemented normal birds than the normal birds not supplemented with *W. somnifera.* The higher weekly body weight gain for *W. somnifera* supplemented group and the insignificant difference in the weekly feed consumption substantiated the result. Wheeler (1994) reported that Zeetress® supplemented birds exhibited improved feed conversion ability. Mishra and Singh (2000) also noticed increased feed conversion efficiency in broiler chicken supplemented with 0.5 per cent *W. somnifera* / kg of feed than 1 per cent *W. somnifera* / kg of feed. Randae *et al.* (2002 a) indicated a similar improved feed efficiency in birds supplemented with Immuplus®. Samarath *et al.* (2002) also reported better feed conversion efficiency to *W. somnifera* supplemented broiler chicken. The present study also revealed a similar trend but birds supplemented with 10 g *W. somnifera* in feed had more efficiency than those supplemented with 5 g *W. somnifera*/ kg feed.

Birds subjected to overcrowding had the lowest feed efficiency, compared to normal birds. The feed efficiency was better in stressed birds fed with 10 g *W. somnifera* / kg of feed than stressed birds supplemented with 5 g *W. somnifera* / kg of feed. Cravner *et al.* (1992) and Gill and Sharma (1992) opined that increasing the stocking density decreased the feed efficiency and this was in agreement with the present study. Rajeshwari *et al.* (2001) reported that supplementation of Zeetress® helped to minimize the stress and had positive effect on feed conversion. Wheeler (1994) reported that extracts of *W. somnifera* ameliorated different undesirable effects of stress in chicken. It could be concluded that supplementation of *W. somnifera* in the diet of broiler chicken improved the feed efficiency as well as enhanced the feed efficiency in broiler chicken subjected to overcrowding stress.

5.3 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON CARCASS CHARACTERSTICS OF BROILER CHICKEN

5.3.1 Slaughter Weight

The present study showed no significant difference between groups. This finding is in agreement with the results of Samarth *et al.* (2002), where supplementation of 0.5 per cent *W. somnifera* / kg of feed mix in broiler chicken induced similar live body weight as that of non supplemented groups. Birds reared under reduced floor space without supplementation of antistress agent had a lower slaughter weight than 5 g *W. somnifera* / kg of feed and 10 g *W. somnifera* / kg of feed supplemented birds. In the present study the weekly body weight, weight gain and feed efficiency was lower for birds subjected to overcrowding stress and that had resulted in the lowering of slaughter weight in these birds.

Bolton *et al.* (1972) reported that decrease in space allowance in broilers from 0.093 to 0.047 m^2/ bird was accompanied by reduced final live weight. Puvadolpirod and Taxon (2000) concluded that experimental induction of stress by continuous administration of ACTH in chicken also resulted in decreased body weight. *W. somnifera* supplementation had a positive effect on the stressed birds. The body weight was higher in stressed birds supplemented with 5 g *W. somnifera* / kg of feed and 10 g *W. somnifera* / kg of feed. Rajeshwari *et al.* (2001) reported that Zeetress® helped to minimize stress and induced a positive effect on live weight. It could be concluded that supplementation of *W. somnifera* in stressed birds improved the slaughter weight, since these birds showed improved weekly body weight, weight gain and feed efficiency.

5.3.2 Carcass Weight

The present study revealed no significant difference in the carcass weight between normal birds and normal birds supplemented with 5 g *W. somnifera* / kg of feed and 10 g *W. somnifera* / kg of feed and this is not in agreement with the finding of Javed *et al.* (2009), where the aqueous extract of several plant

mixtures (one of the ingredients was *W. somnifera*) increased the carcass weight on broiler chicken. Samarth *et al.* (2002) reported that *W. somnifera* supplemented broilers had a higher carcass weight which too did not agree with the present results.This could be due to difference in the strains used for the study.

The birds kept on reduced floor space had a lower carcass weight compared to those kept on optimum floor space. According to Cravner *et al.* (1992) and Feddes *et al.* (2002), broilers subjected to overcrowding stress had lower body weight and carcass weight. This was attributed to stress and these results agreed with the present study. Al-Batsman and Hussein (1999) also observed that the stress induced by high temperature reduced carcass weight. The stressed birds supplemented with *W. somnifera* had a higher carcass weight than the non supplemented stressed birds, which suggested that *W. somnifera* had alleviated the stress to a certain extent. Wheeler (1994) reported that *W. somnifera* supplementation reduced stress due to overcrowding and thus improved the carcass weight. The present study concluded that supplementation of *W. somnifera* in the feed reduced the stress related decrease in carcass weight.

5.3.3 Giblet Weight

In the present study Giblet weight was numerically higher in normal birds than normal birds supplemented with 5 g *W. somnifera* / kg of feed and 10 g *W. somnifera* / kg of feed, but there was no significant difference between them. This indicated that *W. somnifera* supplementation at 5 g/ kg of feed and 10 g/ kg of feed did not interfere with the growth of the organs like heart, gizzard and liver which parallel the non significant increase observed with respect to live weight and weekly body weight in these birds. There was no significant difference in the weight of giblet between stressed birds and unstressed normal birds. The birds subjected to floor space reduction had a numerically lower giblet weight compared to 5 g and 10 g *W. somnifera* supplemented birds in stressed groups. The present study agreed with the findings of Gill and Sharma (1992), where the birds on 1 sq.ft /bird had a higher giblet weight compared to birds on 0.75 sq.ft /

bird. The weight of giblet was numerically higher in stressed birds with 10 g *W. somnifera/* kg feed than those fed with 5 g *W. somnifera/* kg feed. Supplementation of *W. somnifera* did not interfere with the growth of certain internal organs, both in normal and chicken subjected to overcrowding stress.

5.3.4 Weight of Bursa

There was no significant difference in the weight of bursa between normal groups and normal birds supplemented with 5 g *W. somnifera* and 10 g *W. somnifera* in feed. The bursal weight was numerically highest in normal birds than 10 g *W. somnifera* / kg of feed and 5 g *W. somnifera* / kg of feed. There was significant reduction in bursal weight in normal stressed birds compared to normal birds. The present study agreed with findings of Puvadolpirod and Taxon (2000) where experimental induction of stress by continuous administration of ACTH in chicken reduced the weight of bursa in chickens.

The stressed birds supplemented with 10 g *W. somnifera* / kg of feed had increased weight of bursa than non supplemented stressed birds. Kolte *et al.* (2007) and Jadhav *et al.* (2008) reported that supplementation of *W. somnifera* improved immune status in immunosuppressed and climatically stressed broiler chicken. The present study revealed that birds supplemented with 10 g *W. somnifera* in feed could improve the immune status by stimulating bursal growth. Besides, the supplementation of *W. somnifera* reduced the stress related reduction of bursal weight in overcrowdedly housed broiler chicken.

5.3.5 Weight of Spleen

The result of the present study revealed that there was no significant difference between normal chicken and those supplemented with *W. somnifera.* The present study is not in agreement with the findings of Das and Chatterjee (1994), where administration of Stresszee® in rats resulted in an increase in lymphocyte population, which potentiate cellular and humoral components of immune system. Anand and Kuttan (1995) Davis and Kuttan (1997) also

reported a significant increased in spleen weight in *W. somnifera* administrated mice than non administrated ones. Davis and Kuttan (2000) also reported that *W. somnifera* treatment enhanced antibody titre and number of antibody producing cells in the spleen of mice. The present study agreed with the finding of Mejo (2006), where no significant difference in weight of spleen was observed in chickens supplemented with Gooseberry and Indian gallnut.

There was no significant difference in weight of spleen between stressed and non stressed birds. The present study agreed with Puvadolpirod and Taxon (2000), where stress induced by continuous administration of ACTH decreased the weight of spleen. In the present study also there was numerically decreased weight of spleen in stressed birds compared to normal birds. It could be summarized that the supplementation of *W. somnifera* in feed reduced the stress related decrease in weight of spleen in broiler chicken.

5.3.6 Weight of Adrenals

In the present study there was no significant difference in the weight of adrenals in normal birds. The normal birds and stressed birds had a significant difference in weight of adrenals, where there was an increase weight of adrenals in stressed birds. This was due to the physiological response to stress for increased corticosteroid hormone synthesis. Among the birds in stressed groups, birds without *W. somnifera* supplementation had a significantly increased weight of adrenal glands, even though they had least carcass weight and slaughter weight. According to Nayanthara *et al.* (2009) chronic stress caused a significant increase in the weight of the adrenal glands in adult albino rats, which was similar to the findings in the present study in stressed broiler chicken. According to Singh *et al.* (1982), *W. somnifera* was adaptogenic and prevented stress induced increase in weight of adrenals in rats. According to Bhattacharya and Ghosal (1994), administration of Zeetress® reduced the stress induced increase in weight of adrenals in rats. In the present study too *W. somnifera* supplementation reduced the stress induced increase in weight of adrenals. The study concluded that

W. somnifera induced antistress effect by reducing the stress induced hypertrophy of adrenal glands.

5.4 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON HAEMATOLOGICAL PARAMETERS OF BROILER CHICKEN

Haematological parameter such as Hb, VPRC, TEC, TLC, MCV, MCH and MCHC reflects the normal health status of the animal/ bird.

5.4.1 Haemoglobin (Hb) Concentration

The study revealed that there was no significant difference in Hb concentration between normal birds and birds supplemented with *W. somnifera* in feed. There was no significant increase in Hb concentration, but an age related increase in Hb concentration was noticed in all these birds. Akotkar *et al.* (2007)., Daisy *et al.* (2008)., Oyagbemi *et al.* (2008) and Singh *et al.* (2009) reported a significant increase in Hb concentration in *W. somnifera* supplemented birds than normal birds with a concomitant increase in TEC and PCV. Wajari *et al.* (2007) also did not get any significant change in Hb value after *W. somnifera* supplementation in broiler chicken.

There was no significant difference in Hb concentration between stressed normal birds and the normal birds except for the first day after stress, where the Hb level was significantly low in stressed birds. According to Bedanova *et al.* (2006) reduction in floor space increased the Hb concentration in broiler chicken. But Emre *et al.* (1991)., Reddy (2003) and Karthiayini and Philomina (2008) observed that the overcrowding stress would not significantly affect the Hb concentration on the broiler chicken. In the present study too there was no effect of stress on Hb concentration. Reddy (2003) also reported that supplementation of Zeetress® had no effect on Hb concentration in overcrowded stressed broilers. Mejo (2006) also reported that heat stress did not significantly affect the Hb concentration of birds. The Hb concentrations of *W. somnifera* supplemented stressed birds were not significantly different from those stressed birds not

supplemented with *W. somnifera*, but were numerically higher than the stressed birds. The supplementation of *W. somnifera* did not affect the Hb synthesis in normal and stressed birds.

5.4.2 Volume of Packed Red Cells (VPRC)

Hematocrit represents the percentage of total blood volume occupied by the erythrocytes. Its determination is importance in the detection and follow up of anemia and polycythemia. The present study revealed that there was no significant difference in VPRC value between normal groups supplemented and not supplemented with *W. somnifera*. The present study did not agree with findings of Akotkar *et al.* (2007) and Wajari *et al.* (2007), where the VPRC value increased in normal birds supplemented with 0.5 to 1.25 per cent *W. somnifera* in feed.

There was no significant difference in the VPRC values between normal birds and normal birds subjected to overcrowding stress. Emre *et al.* (1991) revealed that population density range of 10, 14, 18 and 20 chicks /m^2 did not affect the VPRC at 40 to 49 days of age. Reddy (2003) also could not observe any a significant change in the value of VPRC in broiler chicken at stocking density of 22 chicks/m^2. Mejo (2006) reported that heat stress did affect the VPRC value. Karthiayini and Philomina (2008) also reported that overcrowding stress induced by reducing the floor space by 50 per cent did not produce any significant difference in VPRC values. The present study also had similar findings. The supplementation of *W. somnifera* to stressed birds did not cause any significant change in VPRC from the non supplemented stressed birds and the values were similar to normal birds. Reddy (2003) also stated that supplementation of Zeetress® to overcrowded and stressed broilers would not affect the VPRC values.

5.4.3 Total Erythrocyte Count (TEC)

In the study there was no significant difference in the TEC between normal birds and normal birds supplemented with *W. somnifera* in feed. Wanjari *et al.* (2007) and Samarth *et al.* (2003) noticed a significant increase in TEC in *W. somnifera* supplemented birds. The present study is not in agreement with the above findings. The difference in sex and strain of the birds used for the study might have contributed to this variation.

At the end of the study a significant difference in TEC was noted between normal birds and stressed birds. The normal birds in stressed group had a significantly lesser TEC than normal unstressed birds. According Bedanova *et al.* (2006), the reduction of floor space during crating decreased the total erythrocyte count. Bedanova *et al.*(2007) also reported that acute heat stress reduced the TEC *in broiler chicken.* But Reddy (2003) and Karthiayini and Philomina (2008) did not get any significant difference in TEC between unstressed birds and stressed birds. Stressed birds supplemented with 5 g and 10 g *W. somnifera* in feed had a significantly higher TEC than non supplemented stressed birds and this could be due to the amelioration of stress related decrease in TEC. It could be concluded that even though *W. somnifera* did not have any effect on TEC in normal birds and it negates the stress related fall in erythropoiesis in stressed broilers.

5.4.4 Total Leukocyte Count (TLC)

The degree of rise in leukocytes depends on the type and severity of the infection and the response of the body. Increase in total leukocyte count is known as leukocytosis and a decrease as leukopenia. The present study revealed that there was a significant difference in total leukocyte count between normal birds and normal birds supplemented with *W. somnifera* in feed. An age related increase in TLC between days was also noticed. The birds supplemented with 10 g *W. somnifera* / kg of feed had a higher leukocyte count compared to normal birds and normal birds supplemented with 5 g *W. somnifera* / kg of feed.

WBC production. There was a dose depended increased production of leukocytes with *W. somnifera* supplementation. Though the low dose (5 g/ kg feed) induced a significant positive effect on TLC up to 5 weeks of age, the higher dose (10 g/ kg feed) could still sustained this effect till the end of the study. According to Ziauddin *et al.* (1996), Davis and Kuttan (2000) addition of *W. somnifera* in feed of mice enhanced the TLC. Dhote *et al.* (2005), Wajari *et al.* (2007), Akotkar *et al.* (2007) reported that *W. somnifera* supplemented in feed significantly increased total leukocyte count in chicken. This is in agreement with the present study.

Except for the third day of stress, on all other days of stress, the TLC count in stressed birds were significantly lower than normal birds. Elevation of corticosteroid level during stress could have caused decrease in the lymphocyte. Wheeler (1994) Vecerek *et al.* (2002) and Mashaly *et al.* (2004) reported leucopenia in heat stressed birds. Mejo (2006) also reported that heat stress induced severe leukocytopenia associated with significant reduction in absolute lymphocyte count. But Karthiayini and Philomina (2008) did not observe any significant variation in TLC due to overcrowding stress in broiler chicken. Reddy (2003) also could not observe any significant difference in TLC count in floor space reduced group supplemented with Zeetress®. Through out the study stressed birds supplemented with *W. somnifera* had a higher TLC than stressed birds not supplemented with *W. somnifera.* But the value came to a significant level only after 12 days of stress. Wheeler (1994) and Das (1994) showed that Zeetress® ameliorated undesirable effects of stress in chicken. Chatterjee (1994b) indicated that stress induced leukocytosis was reduced in mice treated with Streszee®. It could be concluded that the supplementation of *W. somnifera* could improve the TLC both in normal and stressed chicken.

5.4.5 Mean Corpuscular Volume (MCV)

The mean corpuscular volume (MCV) is one of the standard red blood cell indices. MCV is important in the differential diagnosis of anemia. The result

showed that there was no significant difference in MCV value in normal birds and normal birds supplemented *W. somnifera.* Oyagbemi *et al.* (2008) reported that supplementation of *W. somnifera* to broiler chicken for 30 days did not affect the MCV values. The result of the present study is in agreement with the earlier work. Stressed birds not supplemented with *W. somnifera* had a higher MCV than normal birds. This is due to the significant reduction in TEC due to stress in these birds.

Reddy (2003) and Karthiayini and Philomina (2008) reported that overcrowding stress did not affect the MCV value. Though there was a significantly higher MCV value in stressed birds supplemented with *W. somnifera*, when compared to non supplemented stressed birds, it was similar to control birds. Aengwanich (2007) reported that broilers maintained at higher environmental temperature had increased MCV than control birds. It could be summarized that *W. somnifera* supplementation did not affect the MCV in normal birds and prevented the stress related increase of MCV in normal birds.

5.4.6 Mean Corpuscular Haemoglobin (MCH)

Mean corpuscular hemoglobin, is the amount of hemoglobin in a red cell. The MCH is a calculated value derived from hemoglobin and TEC. Though there was a variation in MCH between normal birds for the first three days of stress, this was not evident on later days. At six weeks of age there was no significant difference in the MCH among the normal birds supplemented and non supplemented with *W. somnifera.* The result of the present study was agreement with the findings of Oyagbemi *et al.* (2008) where no change in MCH was observed in growing cockerels after 30 and 60 days of Stresroak® administration.

There was a significantly higher MCH for non supplemented stressed birds compared to normal birds. This is a reflection of the reduced TEC in stressed chicken. The MCH of the stressed birds supplemented with *W. somnifera* had similar value with the normal birds. The result of the present study did not agree with findings of Reddy (2003) and Karthiayini and Philomina (2008), where

overcrowding stress did not significantly affect the MCH value. From the result of the study, it might be concluded that 1/3rd reduction of stocking density (372 cm^2/bird) significantly affect MCH of birds on stress and that *W. somnifera* supplementation did not affect the MCH in normal birds.

5.4.7 Mean Corpuscular Haemoglobin Concentration (MCHC)

Mean corpuscular hemoglobin concentration, is the average concentration of hemoglobin in a given volume of blood. The MCHC is a calculated value derived from the measurement of Hb and the VPRC. The overall result indicated that supplementation of *W. somnifera* did not affect the MCHC in broilers. Oyagbemi *et al.* (2008) also reported that supplementation of *W. somnifera* for 60 days had no effect on MCHC. Reddy (2003) also reported that there was no significant difference in MCHC in normal birds supplemented with Zeetress®. In the present study too supplementation of *W. somnifera* to broilers had no effect on MCHC.

The MCHC did not show any significant difference between normal birds and stressed birds. According to the reports of Reddy (2003), overcrowding stress had no significantly effect on MCHC of six and eight week old broiler chicken. Similarly Karthiayini and Philomina (2008) also did not notice any variation in MCHC in birds subjected to overcrowding stress. The present study also agrees with the earlier workers. Reddy (2003) also reported that supplementation of Zeetress® did not affect the MCHC of birds subjected to stress by reduction of floor space. In the present study too there was no affect on MCHC by supplementation of *W. somnifera* to stressed birds. It could be concluded that *W. somnifera* did not affect the MCHC of normal and stressed birds.

5.4.8 Heterophil Lymphocyte Ratio (H: L)

It has been established that the number of lymphocyte decreased and the number of heterophils increased in response to stressors and increased corticosterone in chicken feed. Broilers exposed to stress showed an increase in

heterophil and decrease in lymphocyte, which lead to increase in H:L. The H:L of about 0.2, 0.5 and 0.8 characterised low, optimum and high levels of stress respectively (Gross and Siegel,1983).

The result showed that there was no significant difference in H:L between normal birds with supplemented and non supplemented with *W. somnifera* upto sixth day of stress. But there was a significant difference from ninth day of stress onwards, where H:L was highest in normal birds without supplementation of *W. somnifera* than the birds supplemented with 5 g *W. somnifera* / kg of feed and 10 g *W. somnifera* / kg of feed. The birds supplemented with 10 g/ kg had a significantly lower ratio towards the end of the study. The study agreed with findings of Rajeshwari *et al.* (2001), where the H:L was normal in Zeetress® supplemented group. But Singh *et al.* (2009) reported that H:L count decreased in broilers supplemented with herbal formulation and Zeetress® and this is agreement to the present study, where lower H:L was observed in *W. somnifera* supplemented normal birds. In the present study the *W. somnifera* supplementation reduced the H:L due to increased stimulation of immune system contributing to increased lymphocyte.

The significant difference in H:L was observed after twelve days of stress and it is maintained till the end of the study. The birds in stressed group without supplementation of *W. somnifera* had an increased H:L than supplemented birds. The present study agreed with finding of Mc Farlane *et al.* (1989) and Atlan *et al.* (1999), where H: L increased with heat stress in broiler chicks. Kuan *et al.* (1990) reported that elevation of H: L was observed with increased stocking density. Gross and Siegel (1983) suggested that in birds H: L was a good measure of long term stress in chicken. Zulkifli and Sti Nor Azah (2004) also reported that heterophil to lymphocyte ratio was a reliable indicator of avian stress. The stressed group supplemented with 5 g *W. somnifera* / kg of feed and 10 g *W. somnifera* / kg of feed decreased the H:L and it is an indicator of reduction of stress. This agreed with the finding of Reddy (2003), where supplementation of Zeetress® suppressed the H:L at sixth and eighth week of age in floor space

reduced group. Similar findings were reported by Rajeshwari *et al.* (2001), where Zeetress® administrated broilers maintained the H:L to normal level and helped to minimize stress. The study indicated that *W. somnifera* supplementation enhanced the H:L in normal and stressed birds. The increase in H:L induced by space reduction stress was ameliorated to by supplementation of *W. somnifera* through its antistress activity.

5.5 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON CERTAIN BLOOD BIOCHEMICAL PARAMETERS OF BROILER CHICKEN

5.5.1 Serum Protein Profile

5.5.1.1 Serum Total Protein

The serum protein level varies according to protein quality of diet and also the physiological conditions of the animals. They constitute enzymes in the body and also act as a energy source. The total protein showed a lot of fluctuation among the control birds. Even after one or two weeks of administration of *W. somnifera* there was no significant increase in protein level in normal supplemented birds than the normal birds. Samarth *et al.* (2003) reported a significant increase of serum protein in birds supplemented with *W. somnifera* @ 0.5 per cent, but no such effect was observed in the present study. Oyagbemi *et al.* (2008) reported that the administration of Stresroak® in growing cockerels increased the total protein. This might be due to the variation in the strain used for the study.

There was a significant decrease in total protein in the normal stressed birds in comparison with normal unstressed birds after six and fifteen days of stress. But this difference was not observed during most of the other periods. Mejo (2006) could not observe any significant difference in total protein in heat stressed cockerels compared to control birds. Karthiayini (2007) also did not get a significant difference in the total protein between stressed and unstressed birds.

Except after twelve days of stress, the total protein was similar for all the stressed birds during the post stress days. Reddy (2003) also opined that supplementation of Zeetress® does not significantly affect the total protein in floor space reduced stressed broilers. It could be concluded that stress induced reduction of serum protein could be evidenced only after two weeks of stress. *W. somnifera* supplemented at 5 g and 10 g / kg of feed did not cause a significant improvement in total protein synthesis both in normal and birds subjected to overcrowding stress.

5.5.1.2 Serum Albumin

Albumin is the most abundant plasma protein and is involved in maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues. Chronic liver disease and kidney disorders decreased the concentration of serum albumin. The result indicated that no significant difference in the albumin level between normal birds and those supplemented with *W. somnifera.* Samarth *et al.* (2003) observed a significant increase in albumin level in *W. somnifera* supplemented broilers. The difference in the present study might due to difference in the sex and strain of the birds used for the study.

There was not much difference in the albumin concentration between stressed and normal birds. Mejo (2006) also reported that there is no significant difference in albumin concentration in heat stressed birds compared to non heat stressed birds. Karthiayini (2007) also could not get any difference in the albumin concentration between normal birds and broilers subjected to overcrowding stress. The supplementation of *W. somnifera* to stressed birds did not reveal any significant variation between the stressed birds supplemented and not supplemented with *W. somnifera.* Reddy (2003) reported that supplementation of Zeetress® to broilers subjected to overcrowding stress had no effect in the serum albumin concentration. Similar to total protein, serum albumin was not affected

by supplementation of *W. somnifera* in normal and birds subjected to overcrowding stress.

5.5.1.3 Serum Globulin

Serum globulins are protein fraction of serum that contains most of the antibodies of the blood. Globulin is also decreased in liver dysfunction and nephrosis. Overall result of the present study revealed that there was not much effect of supplementation of *W. somnifera* on serum globulin, even though a significant fall in the globulin level was observed at the end of the study in birds supplemented with 10 g *W. somnifera* /kg feed. As observed for total protein and albumin, it could be inferred that *W. somnifera* did not affect in serum protein synthesis. As mentioned earlier the positive effect of globulin production observed by Samarth *et al.* (2003) in *W. somnifera* supplemented birds could be attributed to the different strain of the bird used for the study. Oyagbemi *et al.* (2008) also reported an increase in globulin in cockerels supplemented with Stresroak® for one month and the presence of several other herbs along with *W. somnifera* might have contributed to this effect. There was no significant difference between stressed and non stressed birds in the globulin concentration.

The supplementation of *W. somnifera* to stressed birds also did not elicit any significant change in globulin concentration compared to the non supplemented stressed birds. Mejo (2006) also reported that there was no significant difference in serum globulin between normal and heat stressed birds. Karthiayini (2007) reported decreased globulin concentration due to overcrowding stress in broilers, where the floor space reduction was 50 per cent. In the present study only 33 per cent reduction of floor space was induced and this might be the one of the reason for variation. Reddy (2003) also could not observed any change in globulin level between normal stressed birds and stressed birds supplemented with Zeetress®, which is similar to the present study. It could be recommended that *W. somnifera* supplementation does not affect the serum globulin of normal and stressed birds, which was similar to serum total protein and serum globulin.

5.5.1.4 Serum Albumin Globulin Ratio (A:G)

Albumin globulin ratio is an indicator of liver function. A:G increased in excess glucocorticod and decreased in liver dysfunction. Overall results of the study revealed that *W. somnifera* did not induce much change in A:G in normal birds even though a significant reduction was observed on day twelve of stress in 5 g/ kg feed *W. somnifera* supplemented normal birds, which was due to the abarrent fall in globulin during the same period. Samarth *et al.* (2003) observed an increased A:G in *W. somnifera* supplement birds and might be due to the difference in sex and strain used for the study.

The stressed birds and non stressed birds did not show any variation in A:G. This is not in concurrence with finding of Karthiayini (2007), who reported that overcrowding stress increased the A:G in broilers. The absence of any significant change in the A:G in *W. somnifera* supplemented stressed broilers is similar to the observation by Reddy (2003). It could be concluded that *W. somnifera* supplementation did not affect the overall A:G both normal and stressed birds.

5.5.2 Serum Enzymes

5.5.2.1 Serum Alanine Amino Transferase (ALT)

Alanine amino transferase is found in serum and in various bodily tissues, but is most commonly associated with the liver. ALT is the enzymes in the serum represent normal functioning of liver and muscle. The serum ALT level increases in liver disorders and myocardial infarction. The ALT value showed a significant increase in birds supplemented with 5 g *W. somnifera* / kg of feed on ninth and twelfth day of stress and on third day of stress in the birds supplemented with 10 g *W. somnifera* / kg of feed. The rest of days the ALT level was similar or less than the normal birds without supplementation of *W. somnifera* in feed. Oyagbemi *et al.* (2008) reported that Stresroak® supplementation for 30 days did not affect the ALT level in broiler chicken. The present study indicated that

supplementation of *W. somnifera* did not induce a sustained elevation of ALT, a marker of hepatotoxicity. Das and Chatterjee (1994) reported that 30 days supplementation of Stresroak® did not affect the ALT level in rats and that it is not hepatotoxic.

There was no significant difference on the ALT level between stressed birds and normal birds. Karthiayini (2007) also reported the plasma ALT was not significantly affected in stressed chicken subjected to overcrowding. Geetha (1993) and Kamath (1994) revealed that Stresszee® reduced the stress related elevation of ALT in rats but such a significant difference was not observed in this study. The study indicated that overcrowding stress did not significantly affect serum ALT level and that supplementation of *W. somnifera* did not induce hepatocellular damage.

5.5.3 Serum Lipid Profile

5.5.3.1 Serum Total Cholesterol

Cholesterol is the main lipid found in the blood, brain and liver tissue. It is also the precursor of many steroid hormones. The liver metabolizes the cholesterol and it is transported by the blood stream as lipoproteins. The present study indicated no significant difference in serum cholesterol in normal birds and normal birds supplemented with *W. somnifera* in feed. The serum cholesterol level was not influenced by either of the two doses of *W. somnifera* after six weeks of supplementation. Visavadiya and Narasimhacharya (2007) reported that *W. somnifera* when added to diet @ 0.75 / day for four weeks in rats caused no significant change in plasma cholesterol. But supplementation @ 1.5 g/ day reduced the plasma cholesterol in rats. Such an effect was not observed in the present study.

Towards the end of the study stressed birds without *W. somnifera* supplementation showed a significantly higher in total cholesterol than the normal birds. Donkoh (1989) noted that hypercholesteremia in broilers exposed to heat

and cold stress. According to Cetin and Tuncel (1995) the stress symptoms included an increase in the concentration of plasma total cholesterol in broiler chicks of six and eight weeks of age. Based on the earlier reports, hypercholesterolemia observed in the present study also could be considered as a biological response of overcrowding stress. The stressed birds supplemented with *W. somnifera* had a significantly lower cholesterol level than the stressed birds not supplemented with *W. somnifera.* According to Visavadiya and Narasimhacharya (2007) the cholesterol lowering effect of *W. somnifera* is through increased excretion of cholesterol and bile acids through faeces. It could be inferred that even though *W. somnifera* did not much effect the blood cholesterol in normal birds could reduce the stress related hypercholesteremia in broiler chicken.

5.5.4 Serum Glucose

Glucose is the major carbohydrate present in the blood and serves as a primary source of energy. Elevated blood glucose levels are found in hyperadrenalism and certain liver disorders and decreased level found in hypopituitarism and insulinoma. The present study did not reveal any significant difference in serum glucose between normal birds and normal birds supplemented with *W. somnifera* in feed. Samarth *et al.* (2003) reported that broilers supplemented with 0.5 per cent *W. somnifera* in feed would increase blood glucose level. In the present study no such effect could be found and this might be due to difference in sex or strain of the birds.

Stressed birds had a significantly higher glucose level during third and twelfth day of stress. Even though at the end of the study (6 week of age) there was no significant difference in glucose level between stressed normal birds and normal birds, the glucose level was numerically higher in the stressed birds than normal birds. According to Siegel (1961) stress increased the circulating glucocorticoides, which in turn elevated blood glucose concentration in young chicken. Similar observation was noticed by Sawnhey *et al.* (1986), where stress induced hyperglycemia was related to increased adrenaline and suppression of

insulin like activity in man. Karthiayini (2007) observed a numerical increase in glucose level in broiler chicken subjected to overcrowding stress than normal birds. The stressed birds supplemented with *W. somnifera* in feed also expressed a numerically lower serum glucose level than the stressed birds not supplemented with *W. somnifera,* on the days the stressed normal birds expressed a significantly higher glucose level than the normal birds. Geetha (1993) and Kamath (1994) reported that Stresszee® reduced the stress induced higher glucose concentration in rats. Andallu and Radhika (2000) also revealed that *W. somnifera* supplementation caused reduction of blood glucose in type 2 diabetic patients. In the result of the present study too the *W.. somnifera* supplemented birds were having a reduced glucose than stressed birds. It could be concluded that *W. somnifera* did not affect the glucose level in normal birds but reduced the serum glucose in stressed birds.

5.5.5 Serum C - reactive protein (CRP) level

C-reactive protein is a member of the class of acute-phase reactants and its level rises dramatically during stress and inflammatory processes in the body. This increment is due to a rise in the plasma concentration of IL-6. Result of the present study revealed that C-reactive protein increased in stressed birds without supplementation of *W. somnifera* after nine days of stress. Heat stress increased the CRP level in broiler chicken (Mejo, 2006). The results of the present study agreed with the above findings and indicated that stress induces increased C-reactive protein level. The supplementation of *W. somnifera* reduced the CRP level. Kumar *et al.* (2005b) reported that antioxidants reduced the elevated C-reactive protein level in aflatoxicosis in chicken. Mejo (2006) reported that supplementation of gooseberry and Indian gallnut reduced the stress related increase of C- reactive protein in broiler chicken. In the present study the overcrowding stress increased the C-reactive protein level in stressed birds and the supplementation of *W. somnifera* was able to ameliorate the stress related increase of C-reactive protein in broiler chicken.

5.6 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON LIVER LIPID PEROXIDATION AND REDUCED GLUTATHIONE OF BROILER CHICKEN

5.6.1 Lipid Peroxidation (LPO)

Lipid peroxidation level is a direct indicator of oxidative damage of cells and is seen in stress, aging, atherosclerosis and other pathological disorders.

In the present study, there was no significant difference in LPO between normal birds and normal birds supplemented with 5 g *W. somnifera* in feed. The present study agreed with the findings of Pandae and Kar (1997). They evaluated free radical scavenging activity of *W. somnifera* root powder in mice and reported that *W. somnifera* decreased LPO in mice. The supplementation of *W. somnifera* significantly reduced the lipid peroxidation in liver. According to Visavadiya and Narasimhacharya (2007) *W. somnifera* reduced the LPO in hypercholesteremic rats. In the present study the birds supplemented with *W. somnifera* had reduced the LPO in liver indicating free radical scavenging activity.

The stressed birds had a significant higher LPO value than normal birds. This indicated that overcrowding birds resulted in increased free radical production leading to destruction of lipids. Dhuley (1998) evaluated the effect of *W. somniferea* on lipid peroxidation in stress induced a rabbits and mice. Elevation of LPO was observed in rabbits and mice after administration of lipopolysaccharide and *W. somniferea* prevented the rise in LPO. Maini *et al.* (2007) reported that summer stress elevated LPO level in broiler chicken. According to Nayanthara *et al.* (2009), chronic stress increased the LPO in Wistar rats. The results are in agreement with the above findings. The birds supplemented with 5 g and 10 g *W. somnifera*/kg of feed had reduced lipid peroxidation than non supplemented group and thus reduced free radical induce peroxidation. The stressed birds supplemented with *W. somnifera* had a significantly lowered LPO value than stressed non *W. somnifera* supplemented group. Bhattacharya *et al.* (2001) reported that *W. somnifera* glycowithanolides (WSG) normalize the

augmented lipid peroxidase activity in stressed mice. Harikrishnan *et al.* (2008) also reported that *W. somnifera* offered hepatoprotection by decreasing the level of lipid peroxidation products in experimental hyperammonemic rats. The result of the present study agreed with the above findings. It could be concluded that *W.* somnifera posses free radical scavenging activity both in normal and stressed birds and thus ameliorate the stress.

5.6.2 Reduced Glutathione (GSH)

Glutathione is considered to be the master antioxidant of the body and is found in almost all living cells. Body utilizes GSH chiefly for reducing oxidized vitamin C and vitamin E back to their reduced state, to detoxify many toxins, to maintain cellular redox potential and to maintain erythrocytes membrane integrity. Oral supplementation of vitamin C raised the reduced glutathione level. Diminished content of GSH in cells ultimately result in cell death. Oxidative stressors could deplete the GSH level.

In the present study there was significant increase of GSH in normal birds supplemented with *W. somnifera* compared to birds not supplemented with *W. somnifera*. The reduced glutathione was highest in normal birds with supplementation of 10 g *W. somnifera* compared to other two groups. Patania *et al.* (1998) reported that Geriforte, a herbomineral preparation, given at 1 g per cent dose level for four weeks significantly increased, the levels of reduced glutathione in rats compared to control group. According to Yuvaraj *et al.* (2003) the supplementation of 1 per cent spirulina in broiler feed increased the GSH level than control birds.

The stressed birds not supplemented with *W. somnifera* had significantly lower GSH level than non supplemented normal birds. The birds subjected to overcrowding stress without any *W. somnifera* supplementation had a significantly reduced the GSH level than those in stressed birds supplemented with *W. somnifera*. The high level of GSH in *W. somnifera* supplemented stressed birds were similar to those of unstressed birds supplemented with *W. somnifera*.

All birds supplemented with *W. somnifera* birds had a significantly higher level of GSH than normal birds. This clearly indicated that *W. somnifera* supplementation increased GSH in both normal and stressed birds. Kumar *et al.* (2005b) reported that summer stress reduced the liver glutathione in broiler chicken. Liver reduced glutathione was found to be significantly lowered in heat stressed chicken. (Ramnath *et al,* 2007). Kumar *et al.* (2005b) revealed that Zeetress® could ameliorate stress of salinomycin toxicity and thus elevate reduced GSH level. Ramnath *et al,* (2007) also reported that herbal preparation Brahma Rasayana could enhance reduced GSH in heat stressed chicken. The present study revealed that *W. somnifera* supplementation enhanced the GSH in normal birds as well as in stressed birds through antistress activity.

5.7 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON IMMUNOLOGICAL PARAMETER OF BROILER CHICKEN

Stress negatively affects the immune response in animals. After stress there was decrease the IgG and IgM concentration. In the present study the significant difference in HA titre between normal birds and *W. somnifera* supplemented birds were evidenced only on the first day of stress. The antibody titre was higher in normal birds supplemented with *W. somnifera* and this was evident till the end of the study. Result of the present study revealed that *W. somnifera* supplementation (5 g and 10 g / kg of feed) increased circulating antibody titre in chicken which is in agreement with the reports of Das and Chatterjee (1994) and Ziauddin *et al.* (1996). They opined that administration of Stresszee® and *W. somnifera* respectively potentiated the cellular and humoral components of immune system in rats and mice. Davis and Kuttan (2000) reported that administration of *W. somnifera* stimulated the immune system in rats by enhancing the antibody titre and antibody producing cells of the spleen. Singh *et al.* (2009) reported that administration of Immuplus® enhanced TLC and absolute lymphocyte count in birds vaccinated for New Castle Disease and Infectious bursal disease. Hindustani

and Singh (2006) also reported that Zeetress supplementation enhanced the immune response in IBD vaccinated birds.

More than one week of stress lowered HA titre of stressed birds than normal birds, even though the values were not different statistically. Guo and Liu (1998) concluded that heat stress suppressed immunological competence in broiler. Ombasilar and Aksoy (2004) reported that increase in the cage density decreased the antibody titre to SRBC. Mejo (2006) reported that the heat stressed birds had a low profile of humoral immune response indicated by low HA titre. Zahra *et al.* (2008) reported that heat stress led to a significant decrease in antibody level. The present study too the HA titre was reduced in stressed birds which indicated the suppression of immune system by stress. Stressed birds supplemented with *W. somnifera* had a higher HA titre, indicated potentiation of immune system by *W. somnifera*. According to Khan *et al.* (2004) proliferation of T-lymphocyte was evinced by the administration of *W. somnifera* in mice under stress. Jadhav *et al.* (2008) reported that supplementation of *W. somnifera* enhanced HA titre in heat stressed birds and also ameliorated the stress induced suppression of immune system in birds. It could be concluded that supplementation of *W. somnifera* enhanced the immune system in normal birds.

5.8 DIETARY SUPPLEMENTATION OF *WITHANIA SOMNIFERA* ON GASTROINTESTINAL ENZYMES OF BROILER CHICKEN

5.8.1 Proventricular Protease

Protease refers to a group of enzymes whose catalytic function is to hydrolyze peptide bonds of proteins. They are also called proteolytic enzymes or proteinases. Proteases differ in their ability to hydrolyze various peptide bonds. The present study revealed that there was no significant difference in protease activity of normal birds and supplemented birds. Protease activity was numerically higher in the normal birds supplemented with *W. somnifera* and the highest was in normal birds supplemented with 10 g *W. somnifera*. The increase in the enzyme activity coincided with increased feed consumption in normal birds

supplemented with *W. somnifera*. According to Shisodiya *et al.* (2008) supplementation of *W. somnifera* in feed significantly increased the feed consumption and this could be the reason for elevated enzyme activity.

There was no significant difference between normal birds and stressed birds on the proventricular protease activity. There was no significant difference between *W. somnifera* supplemented and non supplemented stressed birds. The results revealed that birds subjected to overcrowding stress without supplementation of *W. somnifera* in feed had the lowest enzyme activity than supplemented birds. The overcrowding reduced the feed consumption and it resulted in the decreased enzyme activity in broilers. Kuan *et al.* (1990) reported that increasing stocking density (stress) reduced the feed consumption in broiler chicken. It could be concluded that protease activity parallels with the feed consumption and *W. somnifera* supplementation increased feed consumption leading to increase the enzyme activity.

5.8.2 Intestinal Amylase

An amylase is an enzyme that breaks starch down into glucose. The present study revealed that significant difference in amylase activity between normal birds and normal birds supplemented with *W. somnifera* in feed. The amylase activity was significantly higher in normal birds supplemented with *W. somnifera* in feed than non *W. somnifera* supplemented normal birds. Akotkar *et al.* (2007) reported that *W. somnifera* in feed (above 0.5 per cent and below 1.25 per cent) improved the feed consumption in broiler chick. Feed consumption increased enzyme activity in supplemented birds. Platel and Srinivasan (2004) reported that rat pancreatic amylase activity was elevated upto 96 per cent by curcumin compared to control group. Namagirilakshmi (2005) reported that the highest intestinal amylase activity for broilers treated with curcumin powder than control group.

The normal birds showed no significant difference with stressed birds on the amylase activity. The birds subjected to floor space reduction without

supplementation of *W. somnifera* had the numerically lowest enzyme activity. In stressed birds the enzyme activity was decreased but stressed birds supplemented with 10 g *W. somnifera* / kg feed had a significantly increased enzyme activity than non supplemented stressed birds. In the present study birds subjected to overcrowding had decreased enzyme activity due to decreased feed consumption. Shivakumar *et al.* (2004), Dozier *et al.* (2005) and Samale *et al.* (2008) reported that broiler chicken subjected to overcrowding stress reduced the feed consumption and this might be the reason for the lesser enzyme activity. It could be summarized that stress reduced feed consumption and decreased amylase activity and that the supplementation of *W. somnifera* improved the feed consumption and increased the enzyme activity.

5.8.3 Pancreatic Lipase

Lipase is a water-soluble enzyme that catalyzes the hydrolysis of ester bonds in water insoluble, lipid substrate. Lipases perform essential roles in the digestion, transport and processing of dietary lipids. In the present study there was significant increase in enzymė activity for normal birds supplemented with 10 g *W. somnifera* compared to the other the normal group of birds. Normal birds with 10 g *W. somnifera* in feed had the highest lipase activity, than 5 g supplemented ones. This was supported by the increased feed intake by *W. somnifera* supplemented birds. Platel and Srinivasan (2001) stated that pancreatic lipase was stimulated in rats by ajowan up to 26 per cent. A single oral dose of mint leaf and garlic oil increased lipase activity by 4-6 folds and 2.5-5 folds, respectively in rats (Sharatchandra *et al.*, 1995). Namagirilakshmi (2005) reported that broilers fed turmeric powder decreased the lipase activity compared to control group. According to Shisodiya *et al.* (2008) *W. somnifera* supplemented broiler chickens had an improved feed intake. In this present experiment there was increased feed consumption in *W. somnifera* supplemented birds and this could have contributed to the increased lipase activity in the *W. somnifera* supplemented groups.

The lipase activity decreased in non supplemented stressed birds than the normal birds. In stressed birds there was significant difference between the three groups. Stressed birds supplemented with *W. somnifera* had a significantly increased enzyme activity than non supplemented birds. This might be due to the reduction in stress and increased feed consumption. Kuan *et al.* (1990) found out that increasing stocking density reduced the feed consumption in broiler chicken. In the present study also the feed consumption was lesser in stressed birds and this could lead to decrease the lipase activity. Wheeler (1994) reported that supplementation of *W. somnifera* reduced the stress in intensively housed chicken, improved feed consumption and thus increased the live body weight. It could be inferred that lipase activity was improved by *W. somnifera* supplementation and that stress related decreased feed consumption leading to decreased lipase activity could be attenuated by supplementing *W. somnifera* to birds.

To conclude it could be inferred that supplementation of *W. somnifera* would not affect the normal growth and haematological parameters of broiler chickens. The activities of liver enzymes ALT indicated that *W. somnifera* was not hepatotoxic to broilers. *W. somnifera* supplementation improved the performance of various body systems. The birds subjected to 1/3 rd reduction of floor space were under stress. This was substantiated by the increased H:L, glucose, cholesterol, adrenal weight, CRP and reduced TEC, TLC and total proteins. Stressed birds were subjected to free radical oxidative damage, which was reflected by the decreased GSH and increased LPO levels. Analysis of TLC, H:L, HA titre and C-reactive protein indicated that there was a marked immunosuppression in stressed broiler chicken. Based on the improvement of certain physiological parameters, which were affected by stress, it could be concluded that *W. somnifera* supplementation would ameliorate stress related oxidative damage, immunosuppression and variation in the physiological systems in broiler chicken.

Further, dose related studies are required to determine the minimum and maximum levels of supplementation of *W. somnifera* to improve production and reduce stress in broiler chicken.

Summary

6. SUMMARY

The study was with the objectives of evaluating the antistress and growth promoting effects of *Withania somnifera* in broiler chicken based on the variation of certain physiologico-biochemical and production parameters. Sixty day old broiler chicks (Vencob) procured from a commercial hatchery were reared under standard managemental condition. They were randomly divided into six groups (G1 to G6) with ten birds in each group. The study was conducted upto sixth week of age. The birds in various groups were as follows, G1- Normal , G2- Normal +5 g *W. somnifera* per kg feed, G3- Normal + 10 g *W. somnifera* per kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* per kg feed, and G6- Stressed + 10 g *W. somnifera* per kg feed.

Production parameters such as body weight, weekly weight gain, feed consumption and feed efficiency were analyzed on weekly basis. All the birds were immunized with 1ml of 7 per cent sheep red blood cells intravenously 5 days before subjecting to stress, for the evaluation of immune status. Stress was induced from four weeks to six weeks of age by reducing the floor space for a bird by $1/3^{rd}$ ($372 cm^2$/bird) of the optimum requirement ($1116 cm^2$/bird).

Blood samples were collected from wing vein, five days and one day before the induction of stress as well on day one, three, six, nine, twelve and fifteen days after the induction of stress. Haematological parameters such as total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin (Hb), heterophil lymphocyte ratio (H:L), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and volume of packed red cells (VPRC) were determined. Serum was utilized for analysis of total protein, albumin, globulin, glucose, C-reactive protein (CRP), alanine amino transferase (ALT) and total cholesterol. The A:G was derived. The immunological status was studied by haemagglutinin assay (HA). Tissue from liver was used for estimating tissue lipid peroxidation and reduced

glutathione levels. The mucous membrane of proventriculus and duodenum were used for protease and amylase estimation respectively. The whole pancreas was homogenized for lipase estimation. Slaughter weight, carcass weight, giblet weight and weight of spleen, bursa and adrenals were also studied.

The highest weight gain was observed in normal birds supplemented with 10 g *W. somnifera.* There was no significant reduction in body weight in stressed birds compared to supplemented stressed birds. The supplementation of *W. somnifera* reduced the stress related lowering of body weight in broiler chicken. Considering the overall feed efficiency, the feed efficiency was higher for normal birds supplemented with 10g *W. somnifera* level. Dietary supplementation of *W. somnifera* improved the feed efficiency in stressed birds.

The supplementation of *W. somnifera* improved the slaughter weight in stressed birds. *W. somnifera* supplementation improved carcass weight in stressed birds and reduced the stress related decrease in carcass weight. The birds subjected to floor space reduction and not supplemented with *W. somnifera* had a lower giblet weight compared to *W. somnifera* supplemented stressed birds. The stressed birds supplemented with *W. somnifera* had increased weight of bursa than non supplemented stressed birds. There was no variation in weight of spleen between any groups. The dietary supplementation of *W. somnifera* reduced the stress induced elevated adrenal gland weight in stressed birds.

There was no significant difference in Hb and VPRC between stressed, normal and *W. somnifera* supplemented birds. Stressed birds supplemented with *W. somnifera* had a significantly higher TEC and TLC than stressed birds without supplementation. Stressed birds not supplemented with *W. somnifera* had a higher MCV than normal birds, due to decreased TEC. The MCH value of stressed birds supplemented with *W. somnifera* was similar to that of normal birds. The MCHC value did not show any significant difference between normal, stressed and *W. somnifera* birds.

The stressed birds without supplementation of *W. somnifera* had a significantly higher H:L than normal birds. The supplementation *W. somnifera* reduced the H:L in stressed birds, through its antistress activity.

The stressed birds supplemented with *W. somnifera* had a numerically higher total serum protein than stressed birds without supplementation. Albumin level showed no significant difference between normal and stressed birds. Serum globulin was not much affected in normal birds supplemented with *W. somnifera.* There was no significant difference in serum globulin between normal and stressed birds without supplementation of *W. somnifera.* Albumin globulin ratio was not significantly different between normal birds and normal birds supplemented with *W. somnifera* in feed. There was also no significant difference in A:G. between non supplemented stressed birds and supplemented stressed birds.

The overcrowding stress did not significantly affect the ALT value and the supplementation of *W. somnifera* did not establish a sustained elevated ALT level indicative of hepatotoxicity.

Stressed birds not supplemented with *W. somnifera* had higher serum cholesterol than normal birds. Dietary supplementation of *W. somnifera* in stressed group reduced the total cholesterol concentration.

Glucose level was numerically higher in the stressed birds than normal birds. *W. somnifera* supplementation reduced the stress induced hyperglycemia in broiler chicken.

The supplementation of *W. somnifera* reduced the CRP level in stressed birds, indicating its antistress activity.

In normal birds LPO was reduced and GSH level was increased by the supplementation of *W. somnifera.* The stress induced elevation of LPO and

reduction of GSH were ameliorated in stressed birds through supplementation of *W. somnifera.*

The antibody titre was higher in normal birds supplemented with *W. somnifera.* Stressed birds supplemented with *W. somnifera* had a higher HA titre than non supplemented stressed birds. Supplementation of *W. somnifera* improved the immune status of normal birds, as well as in the immunosuppressed stressed birds.

Proventricular protease, intestinal amylase and pancreatic lipase activities were numerically higher in the normal birds supplemented with 10 g *W. somnifera* when compared to normal birds. Birds subjected to overcrowding stress without supplementation of *W. somnifera* in feed had the lowest enzyme activities than supplemented birds.

The result of the present study revealed that supplementation of *W. somnifera* would not affect the normal growth and haematological parameters of broiler chickens. The supplementation of *W. somnifera* improved the gastrointestinal enzymes in normal birds. The activities of liver enzymes ALT indicated that *W. somnifera* was not hepatotoxic to broilers. The birds housed in reduced floor space were under stress and it was substantiated by increased H: L, CRP, total cholesterol, glucose, adrenal weight and decreased TEC, TLC and total proteins. Analysis of TLC, H:L, HA titre and C-reactive protein indicated that there was a marked immunosuppression in stressed broiler chicken and *W. somnifera* has immunomodulating effect in stressed birds. In stressed chicken it reduced the increased TEC and MCV. The birds were subjected to oxidative damage due to stress, which was reflected by increased LPO and decreased GSH levels. The supplementation of *W. somnifera* ameliorated the stress related oxidative damage, immunosupresssion and variations in the physio-biochemical system in broiler chicken.

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EVALUATION OF ANTISTRESS AND GROWTH PROMOTING EFFECT OF ASWAGANDHA *(Withania somnifera)* IN BROILER CHICKEN

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ABSTRACT OF THE THESIS

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ABSTRACT

The purpose of the study was to asses the antistress and growth promoting effect of *Withania somnifera* in broiler chicken. Sixty numbers of day old broiler chicks (vencob) procured from a commercial hatchery were reared under standard managemental conditions. At day old they were randomly divided into six groups (G1 to G6) with ten birds in each group. The study was conducted upto sixth week of age. The birds in various groups were as follows, G1- Normal , G2- Normal +5 g *W. somnifera* per kg feed, G3- Normal + 10 g *W. somnifera* per kg feed, G4- Stressed, G5- Stressed + 5 g *W. somnifera* per kg feed, and G6- Stressed + 10 g *W. somnifera* per kg feed. Production parameters such as body weight, weekly weight gain, feed consumption and feed efficiency were evaluated on weekly basis. All birds were immunized with 1ml of 7 per cent sheep red blood cells intravenously five days before subjecting to stress, for the evaluation of immune status. Stress was induced from four weeks to six weeks of age by reducing the floor space for a bird 1/3rd (372 cm^2/ bird) of the optimum requirement (1116 cm^2/bird).

Blood samples were collected from wing vein, five days and one day before the induction of stress as well on day one, three, six, nine, twelve and fifteen days after the induction of stress. Haematological parameters such as total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin (Hb), heterophil lymphocyte ratio (H:L), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and volume of packed red cells (VPRC) were determined. Serum was utilized for analysis of total protein, albumin, globulin, glucose, C-reactive protein (CRP), alanine amino transferase (ALT) and total cholesterol. The A:G was derived. The immunological status was studied by haemagglutinin assay (HA). Tissues from liver were used for estimating tissue lipid peroxidation (LPO) and reduced glutathione (GSH) levels. The mucous membrane of proventriculus and duodenum were used for protease and amylase estimation respectively. The whole

pancreas was used for pancreatic lipase estimation. Slaughter weights, carcass weight, giblet weight, weight of organs (spleen, bursa and adrenal) were also studied.

The result of the present study revealed that supplementation *W. somnifera* did not affect the normal growth of broiler chicken. There was not much variation in slaughter weight, carcass weight and weight of organs between *W. somnifera* supplemented and not supplemented group. In stressed birds supplementation of *W. somnifera* ameliorated the stress related reduction in organ weight.

Supplementation of *W. somnifera* did not affect the haematological parameters such as Hb, VPRC, TEC, TLC, MCV, MCH, MCHC and H:L in normal birds. Supplementation of *W. somnifera* to stressed birds reduced the stress induced increased TEC, MCV and H:L in birds.

The supplementation of *W. somnifera* did not affect the serum total protein, albumin, globulin and A:G in normal birds. But in stressed birds the increased total protein was decreased by supplementation of *W. somnifera.* The supplementation of *W. somnifera* did not cause a sustained increase in ALT values revealing that it was not hepatotoxic to broilers. Supplementation of *W. somnifera* to stressed birds reduced the stress related hyperglycemia and hypercholesterolemia.

The increased level of C-reactive protein in stressed birds was reduced through supplementation of *W. somnifera.* Supplementation of *W. somnifera* increased the GSH and decreased LPO levels in birds. Supplementation of *W. somnifera* ameliorated the stress related oxidative damage through the reduction of LPO and increase of GSH levels. *W. somnifera* improved the immune status of normal birds as well as that of immunosuppressed stressed birds. Besides, it also improved the activities of gastrointestinal enzymes in normal and stressed birds.