EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON THE PHYSIOLOGICAL PARAMETERS IN BROILER CHICKEN

By

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THESIS

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DECLARATION

I hereby declare that the thesis, entitled **"EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON THE PHYSIOLOGICAL PARAMETERS IN BROILER CHICKEN"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON THE PHYSIOLOGICAL PARAMETERS IN BROILER CHICKEN"** is a record of research work done independently by **Dr. Srinivas Reddy Bellur**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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We, the undersigned members of the Advisory Committee of **Dr. Srinivas Reddy Bellur,** a candidate for the degree of Master of Veterinary Science in Physiology, agree that the thesis entitled **"EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON THE PHYSIOLOGICAL PARAMETERS IN BROILER CHICKEN"** may be submitted by Dr. Srinivas Reddy Bellur in partial fulfilment of the requirement for the degree.

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LIST OF TABLES

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LIST OF TABLES

CONTENTS

LIST OF TABLES

Introduction

LIST OF FIGURES

Introduction

1. INTRODUCTION

Stress is essentially a physiological response of the chicken to an external stimulus or demand requiring adjustments for immediate survival and/or adaptation for long term survival. All physiological stress has the effect of diverting the nutrients from economically important parameters to those physiological functions necessary for immediate survival.

Modern animal husbandry methods are aimed at providing the means to produce the animals and birds as efficiently and economically as possible. Some of the management practices like intensive housing methods of animals and birds, feed restriction in poultry etc. have the potential to experience stress which may adversely affect their performance and well being. It is generally accepted that physiological stress is the major underlying factor that adversely affects economically important parameters such as growth rate, immune response, feed conversion efficiency, fertility, hatchability and livability of commercial poultry stock.

The *General Adaptation Syndrome* (Selye, 1937) is an influential theory in biology and medical sciences, which continues to draw considerable attention from physiologists and psychobiologists. Although the theory opened promising frontiers in human medicine, and animals in their natural state, its implications in the context of veterinary science and poultry production have been a major interest. These implications are particularly true for modern production systems where intense selection for economic traits in concert with increased mechanization and changes in husbandry may result in a variety of stress responses.

Once a stressor has been perceived two distinct pathways involving interlocking physiological reactions are evoked. The first pathway encompasses the sympathetic-adrenomedullary (SA) system, which is responsible for the *flight or fight* mechanism. Although this system may have a dramatic physiological impact but it is of short term. When the biological system fails to cope with the stressor(s) and the behavioural activity is suppressed, the second pathway, the hypothalamic-pituitary-adrenal (HPA) axis is elicited which is associated with Selye's adaptive stage of the *General Adaptation Syndrome*.

Growing concern regarding animal well-being is partly responsible for a renewed interest to reassess human-animal relationship. Review of papers on stress in domestic fowl emphasized the importance of the study on physiological responses and their negative consequences on health and well-being. A better understanding of physiological, biochemical and behavioral constitution of birds to stress would be of considerable value in the development of operational strategies and feeding practices under intensive production system to reduce environmental hazards. Perusal of literature revealed that meagre work has been carried out on physiological and biochemical parameters of poultry especially in Kerala under various stress conditions. Hence it was thought worthwhile to evaluate the effects of induced stress as floor space reduction and feed restriction and ameliorating effects of antistress agents such as ascorbic acid and Zeetress® on certain physiological and biochemical parameters of broiler chicken.

Review of Literature

2. REVIEW OF LITERATURE

One of the major interest to poultry producers are the consequences of biologically costly physiological alterations, which can result from husbandry related stressors such as feed restriction, overcrowding, handling, transportation and vaccination. A better understanding of physiological and biochemical constitution of an animal to stress /antistress agents would be useful in the development of operational strategies to reduce environmental insults and to enhance progress in poultry husbandry. A variety of commercially available antistress agents have been tried in poultry. Although the literature on stress is voluminous, understanding of the stress syndrome remains elusive and suffers from confusion and controversy. The information regarding the physiological and biochemical effects of stress in broiler chicken especially in Kerala conditions are scanty.

2.1 Stress in animals and poultry

Amoroso (1967) mentioned *stress* as a situation that release emergency signals necessary for survival. Physiological *stress* as defined by Freeman (1971) is a complex change that occurs in various plasma or adrenal gland constituents following an adverse stimulus. Fraser *et al.* (1975) defined *stress* as an abnormal or an extreme adjustment in the physiology of an animal to cope up with the adverse effects of its environment and management.

Selye (1980) and Ewbank (1985) used the term *stress* to identify the extreme responses to adverse stimuli, which caused a pathophysiological reaction in the host producing associative changes in behavior, physiology and disease susceptibility. Historically biologists defined *stress* in higher animals as physiological state involving heightened activity of pituitary and adrenal cortex and development of gastric ulcers. Webster's New Collegiate Dictionary (1981) defined *stress* as physical, chemical or emotional factors that cause bodily or mental tensions and may be factors in the cause of disease. Williams (1984) briefed *stress* as anything, which disrupts the stability or homeostasis in a stressor and the reaction of the organism to the stressor in *stress*.

Freeman (1987) cited Blakiston's Gould Medical Dictionary definition of *stress* as any stimulus or succession of stimuli of such magnitude as to tend to disrupt the homeostasis of the organism; when incoordinate the stress may be considered as an injury, resulting in disease, disability or death. Anilkumar and Singh (1991) briefed *stress* in poultry as any condition, which adversely affects life or productivity of poultry in a production system. *Stress* is a matter of degree and the maximum expression in bird's death from fright or shock, even though no physical injury is involved. Zulkifli and Siegel (1995) concluded that in poultry production the term *stress* is commonly used to justify unexplained inferior productivity or pathological status.

2.2 Implications of Stressors in poultry industry

Wolff (1960) suggested that any noxious stimuli or the circumstance which produce the state of stress, could be divided into two extreme types. The physical stimuli such as extremes of temperature or corrosive chemicals, which act directly, produce a predictable physiological response. At the other extreme are those which are not having any direct effect, but exert their influence by acting as signals. Such responses are unpredictable and are associated with behavioral and psychological changes, which involve the highest parts of central nervous system.

Curtis (1983) concluded that stressors common to poultry production were extreme environmental temperature, disease, handling, beak trimming, vaccinations, crowding and inadequate ventilation varied in intensity, duration, time and often occurred concurrently with other stressors. Rees *et al.* (1985) and Zulkifli *et al.* (1993) opined that chicken readily habituate to fasts of moderate duration. Griffin (1989) classified stressors into three categories *viz.* (a) Environmental, which included heat and cold, transportation, novel sounds, odour and taste, (b) Behavioral – overcrowding, hierarchical challenge, weaning and feed restriction, (c) Psychological – capture of wild animals and physical restraint.

Kettlewell (1989) classified stress into various conditions as mental, physical and mixed. He included stress during catching, social group and transportation as the mental stressor and harvesting, loading and carrying inverted into crate as the physical stressor. Many stressors involved mixed factors like transportation and handling, which had both mental (pain, fear, anxiety) and physical (environmental wounds) factors. Harbuz and Lightman (1992) in their review classified stress into acute (cold weather, restraint, laprotomy or surgical stress) and chronic (repeated acute challenges or stimuli).

Saxena (1997) divided stressors into two categories; avoidable (overcrowding, social behavior, poor feeding, toxins in feed, poor ventilation, high ammonia levels, rough and frequent handling, dehydration, starvation) and unavoidable (vaccination, transportation, medication, debeaking, handling, high temperature, lighting, extreme weather, high rate of growth).

2.3 Effect of stress on haematological parameters

2.3.1 Floor space reduction

Kuan *et al.* (1990) reported that broiler fowls on the highest stocking densities were under stress, as indicated by their relatively high H:L ratio from fourth week onwards had reduced performance with higher mortality rate. Cravener *et al.* (1992) found that the seven week old birds reared at a floor space of 0.09 and 0.11 sq.m per bird had significantly higher H:L ratio than those reared at 0.05 and 0.07 sq.m per bird. Patterson and Siegel (1998) reported that cage density treatment had no significant effect on hemagglutinin titers to sheep red blood cell antigen, percentage heterophils, lymphocytes, or the H: L ratio.

2.3.2 Feed restriction

Gross and Siegel (1986) demonstrated that heterophil:lymphocyte (H:L) ratio was reduced after repeated fasting indicating that chicken became habituated. Maxwell *et al.* (1990b) reported that the values of haemoglobin(Hb) concentration, volume of packed red cells (VPRC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume(MCV) were all reduced while the total erythrocyte count was increased significantly when broilers were fed a restricted diet. The mean corpuscular haemoglobin concentration (MCHC) and reticulocyte count did not alter significantly. According to them some of the erythrocyte characteristics were restored within "normal" range upon feed restriction but with a tendency of microcytic normochromic anaemia in feed restricted birds.

Maxwell *et al.* (1990a) reported that in chicken basophils were the only type of white blood cells to increase significantly in number after stress associated with feed restriction. There were more basophils in broilers than layers, in males than in females and in younger than in older fowls. In contrast, there was a decrease in total leucocyte count in fowls on the restricted diet compared to *ad libitum* fed birds. They concluded that stress might be responsible for an increased thrombocyte count with a slightly lowered eosinophil count in the feed restricted fowls. According to them the H:L ratio was not significantly altered by feed restriction. Maxwell *et al.* (1991) further reported that early age feed restriction (six, 10 or 14 days) in birds brought significant increase in H:L ratio with slight increase in basophil count and with reduction in eosinophil count. They also observed that seven weeks old birds had significant reduction in erythrocytes, leucocytes and thrombocytes along with significant increase in the values of MCH and MCV. The birds had macrocytic normochromic anaemia of folic acid deficiency due to feed restriction

Maxwell *et al.* (1992) and Maxwell (1993) concluded that in poultry, heterophilia might be the response from mild to moderate stress but basophilia might be the result after severe stress. Maxwell (1993) has comprehensively reviewed the reliability of H:L ratio as a biological indicator of stress in avian species. Hocking *et al.* (1994) found that feed restriction was associated with lower number of heterophils and monocytes resulting in a lower H:L ratio. Maxwell and Robertson (1995) reported that feed restriction produced significant basophilia in avian species. Although H:L ratio was a reliable indicator of avian stress, yet during extreme stress condition, a heteropenia and basophilia often developed. Prabhakaran *et al.* (1997) observed that fasting of birds for two days significantly increased the H:L ratio from 0.36 ± 0.14 to 0.89 ± 0.18.

Zhou *et al.* (1998) reported that the whole blood viscosity (WBV), total erythrocyte count (TEC) and VPRC were greater during the dark (21.00 and 03.00 h) than during the light period and during the dark period, there were no differences in total leucocyte count (TLC), TEC and VPRC between feed and water restricted groups when compared to that of non restricted groups. There were no light-dark differences in restricted and non restricted groups on total plasma protein concentration or MCV. However, TLC increased linearly with TEC and VPRC and total plasma protein concentration increased, while MCV was decreased.

2.3.3 Other stressors

Shapiro and Schechtman (1949) reported that a single injection of adrenocortical extract in adult fowl caused lymphocytolysis leading to transient lymphopenia. Huble (1955) opined that treatment of cockerels with adrenocorticotropic hormone (ACTH) produced heterophilia, lymphopenia and eosinopenia. Stress caused involution of lymphoid tissue (thymus, spleen and bursa of fabricius) which reduced the number of circulating lymphocytes and increased the number of heterophilic granulocytes (Garren and Shaffner, 1956; Glick, 1967; Siegel, 1980, 1983). Huston (1965) observed that VPRC increased when birds were exposed to cold.

According to Sturkie (1965) mean ESR in birds ranged from 0.5 to 9 mm/h and most of the values were between 1.5 and 4 mm/h. Proliferative response of lymphocytes to concanavalin A (Con A) and lipopolysaccharide (LPS) mitogen has been suggested as the measurement of chicken lymphocyte proliferation (Toivanen and Toivanen, 1973; Hovi *et al.*, 1978; Haq *et al.*, 1996). Corwin and Shloss (1980) reported that vitamin E stimulated the mitogenic response of *Salmonella typhosa* LPS and vitamin E enhanced lymphocyte activity by protecting lymphocytes from lipid peroxidation by its antioxidant activity. Heat stress stimulated the release of corticosterone and catecholamines and initiated lipid peroxidation in cell membranes (Freeman and Crapo, 1982) including membranes of T and B lymphocytes.

Gross and Siegel (1983) observed that the lymphocyte count decreased and the heterophil count increased in response to stressor and also to increasing levels of incorporation of corticosterone in chicken feed and they observed that the H:L ratio was less variable and more reliable indicator of stress than plasma corticosterone values in avian species. Frank Bleecha *et al.* (1984) reported an impaired blastogenic response of stimulated bovine lymphocytes to mitogen, in shipped calves. They justified that the elevated glucocorticoids in stressed calves were responsible to suppress *in vitro* interleukin-2 production by lymphocytes necessary for proliferation, impairing the blastogenic response.

Elicitation of adrenocortical activity in known to precede heterophilia (or neutrophilia) and lymphopenia (Golub and Gershwin, 1985 and Siegel, 1985). Donkoh (1989) reported that changes in physiological status, such as increased rectal temperature, decreased TEC, VPRC and total plasma protein concentration were observed in birds housed at high environmental temperature (30 -35° C). McFarlane *et al.* (1989) reported that monocyte was the only leucocyte to have a significant increase in number when birds were subjected to a continuous sound stress.

Gross (1990) observed that in chicken subjected to sound stress (104 decibels for 30 sec) the H:L ratio began to rise 18 h later reaching a peak value (0.62) in 20 h and returned to the pre-stress values after 30 hours. Direct lysis or apoptosis (Munck and Guyre, 1991) and delay in maturation (Sapolsky, 1992) of lymphocytes due to stress induced increase in corticosteroids might further exacerbate the problem of reduced number of lymphocytes in circulation.

Mitchell *et al.* (1992) reported that in transportation stress of birds the H:L ratio and plasma creatine kinase activity were increased with a decreased eosinophil count. Arun and Lokhande (1994) opined that since the avian erythrocytes biconvex shaped and rouleux formation does not occur and the cells show little tendency to sediment, hence ESR is not an useful test in birds. Quan *et al.* (1995) noticed the lymphocyte count was decreased from 63% at pre-shipping to 46.15% after shipping, while heterophils increased from 24.35% to 41.6%. These could be used as indicators for the diagnosis of shipping stress in commercial broilers.

Haq *et al.* (1996) observed that chicks hatched from hens fed dietary 0.03% alpha-tocopherol acetate had higher bursal and spleenic lymphocyte proliferation when stimulated *in vitro* with tetrahydrofuran or Con A than the control chicks hatched from hens without supplemental alpha-tocopherol acetate. Borges *et al.* (1999) found that heat stress increased the rectal temperature, VPRC, Hb concentration, heterophil and H:L ratio and decreased total erythrocyte count and lymphocyte count. They also suggested that H:L ratio and rectal temperature could be used as a stress index.

Furlan *et al.* (1999) observed acute heat stress in chicken was associated with a decrease in TEC, VPRC, and pCO2 with an increase in blood pH and

pO_2. Lazarevic *et al.* (2000) also recorded a decrease in TLC increase in H: L ratio in broilers when exposed to long term sound stress. Puvadolpirod and Thaxton (2000b) reported that following continuous infusion of ACTH (8 IU/kg/day for seven days), a dose related stress response was observed by a rise in H:L ratio in chicken.

2. 4 Effect of stress on biochemical parameters

2.4. 1. Floor space reduction

Pesti and Howarth (1983) found that plasma corticosterone was significantly higher for male broiler chicks housed on battery brooders kept at 697 and 348 cm^2/bird than when kept at 232 or 116 cm^2/bird. Mashaly *et al.* (1984) reported that corticosterone concentration was consistently higher (2397 pg/ml) in serum of birds housed five per cage than in birds housed three (1654 pg/ml) or four (1853 pg/ml) per cage at 48 and 96 h following housing in cages and weekly thereafter for six week. It was concluded that in order to minimize physiological stress in caged layers more than 387 cm^2 floor space per bird should be provided. Gill and Sharma (1992) reported that cortisol level did not differ significantly between the stocking densities (4.425 ng/ml at1 sq.ft/bird and 3.675 ng/ml at 0.75 sq.ft/bird at six weeks of age). However, the level was significantly elevated at sixth week of age as compared to eighth week of age indicating increased resistance to stress with the advancement of growth.

Cetin and Tuncel (1995) opined that the housing density (10, 14, 18 and 22 chicks/sq.m) had no effect on blood biochemistry of 35 day old birds but the levels of blood glucose, cholesterol and total lipid increased in the older birds (42 or 52 days of age) when housed at 22 chicks/sq.m. It was concluded that the stress symptoms could occur in chicks housed in groups of 22 chicks/sq.m and above.

2.4.2 Feed restriction

A partial recovery in blood glucose level, caused by enhanced gluconeogenesis was noticed in chicken during starvation (Hazelwood and Lorenz 1959). Langslow *et al.* (1970) observed a rise in plasma free fatty acid (FFA) and fall in glucose level during starvation. Sykes (1971) reported that starvation significantly decreased the amount of uric acid nitrogen in the urine (from 84 to 58%) and blood with increased level of ammonia (from 6.8 to 23.0%). Nir *et al.* (1973) reported that there was a decreased in plasma glucose concentration with an increase in FFA concentration during starvation of cockerels and geese for eight days. The fall in plasma glucose was followed by a secondary gradual increase. Refeeding caused a rebound in plasma glucose and restored the FFA to its initial level. Plasma triglyceride was not affected by starvation in these two species.

Nir *et al.* (1975) observed a three-fold increase in plasma concentration of corticosterone upon starving the birds. Freeman *et al.* (1981) recorded feed restricted chicken (reducing feed intake to 75% of *ad libitum* intake from hatching to 8 weeks) were lighter throughout the experiment and after one week of feed restriction plasma corticosterone concentration was 73% greater than control, which decreased progressively and reached the normal range within five weeks. The feed restricted birds were hypoglycemic from second to seventh week and hyperlipidemic throughout the experimental period and there was a negative correlation between plasma glucose and FFA .

Bacon (1986) reported that short-term (up to 44 h) feed restriction in male turkeys resulted in an approximate three fold increase in the concentrations of FFA and an approximate 70% decrease in triglycerides at all ages. Bartov *et al.* (1988) stated that plasma corticosterone level was consistent and at times significantly higher in fasted birds. Katanbaf *et al.* (1989) reported that plasma glucose level was lower for feed restricted females than those fed *ad libitum*. The total plasma protein level was greater in skip two day (STD) feeding than for *ad libitum* and skip one day (SOD) feeding groups where as plasma lipids were higher for *ad libitum* and SOD than STD groups.

Anthony *et al.* (1990) conducted an experiment to study the effect of an overnight fast on secretory profile of growth hormone (GH), insulin and related metabolites such as non esterified fatty acids (NEFA), triglyceride and glucose in eight and nine weeks old male turkeys and found that the plasma levels of GH and NEFA were higher and plasma triglycerides was lower in fasted birds compared to fed birds without any change in plasma glucose. Hocking *et al.* (1993) found that the activity of creatine kinase was lower and the activity of aspartate-transaminase was higher in feed restricted birds. Hocking *et al.* (1994) concluded that feed restriction in broiler breeder females was associated with lower concentration of plasma triglycerides.

Al-Rawashdeh *et al.* (1995) studied the effect of starvation in day old broiler chicken for five days and found that chicken fed or starved had similar values of serum glucose upto day four. At day five, serum glucose value in starved chicken decreased sharply, suggesting a shift from fats to glucose as an energy source. Mean triglycerides value was lower for starved chicken upto day three. In fed chicken serum cholesterol concentration decreased throughout. They concluded that in newly-hatched, feed deprived chicks, metabolism might be different from that in adult birds and mammals.

Huff *et al.* (1996) noticed that in turkeys' serum triglyceride concentration was the most sensitive indicator to either water or feed withdrawal with reduction occurring at 16 h after feed withdrawal. Anthony *et al.* (1999) reported that concentration of plasma uric acid was increased with each consecutive day of fasting in turkeys. Vanderwal *et al.* (1999) reported that feed withdrawal before broilers were transported to processing plant showed a decrease in blood glucose level and an increase in NEFA and liver pH. They also observed that glucose level tended to rise again after long deprivation period. The increase in NEFA was lower in males than females. It was concluded that these blood metabolites were not suitable for use as indicator of duration of feed withdrawal, but the liver pH was found to be more reliable indicator of the length of feed withdrawal. Zulkifli *et al.* (2000b) reported a decreased serum concentration of cholesterol in feed restricted broiler chicken than those fed *ad libitum*.

2.4.3 Other stressors

Rise in blood cholesterol level was suggested to be one of the biochemical markers of stress response (Sayers *et al.* 1945). Huston and Subhas (1969) reported that birds held at 30°C had low total plasma protein concentration in comparison to those at 18°C. Christie and Moore (1972) mentioned the lipid components in the plasma of laying hens as triglycerides (59.7%), phospholipids (31.8%), diglycerides (4.3%), cholesterol esters (2.4%) and feed fatty acids (1.8%). In mammals both physiological and psychological stressors found to increase the plasma glucagon concentration by Bloom *et al.* (1972, 1973) and similar responses have been confirmed in fowl by Freeman, (1976).

There was an increase in circulating level of corticosterone shortly after exposure to a stressor (turkeys: Brown and Nestor, 1973 and El -Halawani *et al.* 1973; Chicken: Edens and Siegel, 1975 and Zachariasen and Newcomer, 1975). El -Halawani *et al.* (1973) have also traced the course of acclimation to a change in environmental temperature and reported that plasma corticosterone returned to its initial concentration after two to three weeks depending on the intensity of stressor.

Ward and Peterson (1973) reported that broilers subjected to chronic heat stress (33 to 35°C for 4 weeks) had significantly lower total plasma protein concentration than those reared at 18-22°C for four weeks. Nir *et al.* (1975) reported that ACTH administration caused a marked (3-7 folds) increase in concentration of plasma corticosterone in intact (I), sham operated (S) and bursectomised (Bs) groups of chicken. The plasma glucose concentration was markedly increased by ACTH administration in the I & S groups after one hour and was almost restored to the control level after 3 hours. The increase was non- specific in Bs group. Upon immersion in cold water there was a substantial increase in blood glucose level in all the groups (I, S & Bs).

Freeman and Manning (1976) have shown that there was a 50% rise in the concentration of plasma FFA within five minutes of handling stimuli and this increase persisted for up to 30 minutes by which time the birds became hypoglycemic. Birds lack carbamyl phosphate syntheatase necessary for synthesis of carbamyl phosphate, which is a precursor for ornithine-urea cycle (Sturkie, 1976). Beuving and Vonder (1978) noticed a substantial increase in the plasma corticosterone concentration following two to five seconds of immobilization. Corticosteriod concentration in the blood was used as a measure of environmental stress and physiological activity in chicken (Eskeland and Blom, 1979 and Siegel, 1980).

Stress-induced hyperglycaemia was generally observed as a consequence of increased adrenaline and possible suppression of insulin like activity (Sawnhey *et al.*, 1986). Donkoh (1989) reported that broilers exposed to 30° and 35°C showed decreased concentration of total plasma protein and an increased blood glucose concentration. Elevated levels of corticosteroids caused a reduction in protein biosynthesis as a result of reduced amino acid transport from the liver, which led to a negative nitrogen balance. There was also a reduction in peripheral utilization of glucose due to high corticosteroid concentration in the blood producing a reduction in metabolic rate (Chatterjee, 1994b).

Deyhim *et al.* (1995) found that heat stress reduced the serum concentration of total protein, albumin, triacylglycerol, uric acid. However, there was an increased level of blood sugar. Latour *et al.* (1996) reported that continuous infusion of ACTH in chicken resulted in the increase of plasma concentration of glucose, cholesterol, triglycrides, very high density lipoprotein (VLDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein(HDL)-cholesterol and corticosterone during the post implantation period. Guo and Liu (1997) opined that serum concentration of insulin, uric acid and triglyceride concentration were lowered where as glucose and cholesterol levels were increased significantly in birds under heat stress.

Puvadolpirod and Thaxton (2000a) observed that plasma concentration of corticosterone, glucose, total plasma protein, cholesterol and triglyceride were increased during ACTH infusion. Puvadolpirod and Thaxton (2000c) further reported that the stress response following continuous infusion of ACTH was with an elevated level of plasma corticosterone by two hours and plasma glucose by 12 hours respectively with a decrease in liver soluble protein content by 12 days.

2.5 Effect of stress on production parameters

2. 5. 1. Floor space reduction

Tomhave and Seegar (1945) found that reduction in floor space resulted in lower body weight, increased mortality and poor feed conversion. However, Siegel and Coles (1958) reported that there was no significant difference in the body weight and feed conversion efficiency of broilers reared at various floor space levels ranging from 0.5 to 1.25 sq.ft/bird. Hansen and Becker (1960) showed that there was no significant improvement in 10^{th} week live weight of male chicks housed at 0.046 m^2/bird when the feeder space was increased from 1.27 to 7.62 cm/bird. Deaton *et al.* (1968) reported that stocking density did not significantly affect the mortality percentage.

Bolton *et al.* (1972) reported that decrease in space allowance 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 0.75 sq.ft/bird. Sulane and Ledaiyc (1976) observed no marked difference in body weight of broilers in cage densities of 0.51 to 0.65 sq.ft/bird.

Zoog (1980) also reported that there was decreased growth rate with increased meat production per unit floor space by increasing the stocking density. Gross and Siegel (1981) observed that birds housed individually with no sight of other birds exhibited superior growth and feed efficiency during the first three months and thereafter they became anorectic, emaciated and flabby.

Pesti and Howarth (1983) observed that body weight gain during the 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. Leeson and Summers (1984) compared the effects of providing 293- cm^2 vs 586 cm^2 per bird and found that the more liberal density resulted in 5-8 % increase in feed intake without relative increase in body weight. They concluded that greater nutrient intake was related to a greater maintenance requirement associated with increased bird's activity.

Mandlekar and Thatte (1986) observed no appreciable difference in body weight of broilers at floor space of 0.48, 0.53, 0.58 or 0.61 sq.ft/bird. Quinones *et al.* (1987) reported decreased growth rate when stocking density in cages increased, without any effect on percentage survival. Sheriff and Kothandaraman (1987) obtained numerically higher mortality percentages in caged broilers from seven to ten weeks of age as the stocking density was increased. Shanaway (1988) found that average feed intake over the whole experimental period declined linearly with densities above 20/m^2; with significant improvement in feed efficiency in the birds housed at high stocking densities. Alribdawi and Singh (1989) reported that no significant effect of stocking density on the body weight of broilers up to four weeks of age reared in groups of 10,15 or 20 birds/ m^2.

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 affecting growth rate up to sixth week. After six weeks, birds at lowest stocking rate had the highest average daily gain (39.2 g) and daily feed consumption (127.0 g) and poorest feed conversion (3.3g feed/g gain). Fowls at the highest stocking density had the lowest average daily gain (32.2 g) and feed consumption (90.6 g) and they concluded that stress associated with overcrowding reduced the performance in broiler fowls.

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. At eighth week of age birds at floor space of 1sq.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 or stocking densities. Mortality was significantly higher on deep litter at 0.75 sq.ft/bird density than on wooden slat system.

Cravener *et al.* (1992) reported that there was no effect of population density treatments on feed conversion at sixth or seventh week of age. Birds housed at 0.07, 0.09 and 0.11 m^2/bird had significantly higher body weight and carcass weight than birds housed at 0.05 m^2/bird indicating stress at higher stocking densities. Narayanankutty and Ramakrishnan (1992) reported that birds reared in California type cages (60 x 45 x 45 cm) at densities three, four and five birds per cage where birds in three birds per cage showed higher body weight, carcass yield, lower mortality with better feed efficiency in birds of five birds per cage.

Patterson and Siegel (1998) reported that pullet body weight was significantly reduced when reared at greater bird densities as early as six weeks of age in one strain of White Leghorn(Delta) and in other (W-36) the body weight was not reduced significantly until 18th week of age. At 18 weeks of age the birds weighed 1210 g (Delta) and 1161 g (W-36) at stocking density of 19 birds/cage where as body weight was 1357g (Delta) and 1215g (W-36) at 10 birds/cage. Feed intake was increased by more than 13 per cent in both strains at lowest cage density treatment but reduced by more than 9 per cent in highest cage density. Feed conversion efficiency was improved at higher densities and poor at lowest densities as compared to the standard.

Imaeda (2000) found that sudden death syndrome SDS mortality was increased at the stocking density of 18 birds/ m^2 in summer and with lower body weight gain and feed intake with no significant change in feed efficiency irrespective of stocking density (12, 15 or 18birds/m^2). Sorensen *et al.* (2000) also observed that high stocking density was associated with reduced live weight, poor walking ability and more foot and hock burns.

2. 5. 2. Feed restriction

Nir *et al.* (1975) demonstrated that there was marked decrease in body weight following three days of starvation. The decrease was less pronounced in sham operated and bursectomised group than intact ones. Early age feed restriction resulted in an improved feed conversion efficiency in broiler chicken with reduced carcass fat (Plavnik and Hurwitz, 1988, 1991; McMurtry *et al.*, 1988). Later Katanbaf *et al.* (1988) were also of the opinion that the body weight of feed restricted birds was less than half of that of *ad libitum* fed chicken by 160 days of age and the mortality was lower for feed restricted birds. Fontana *et al.* (1992) also reported that broilers subjected to early feed restriction had significantly lower mean body weight than control of all ages. According to them feed conversion ratio and weekly body weight gain were significantly lower for feed restricted groups from 0-28 days of age but had significantly higher rate of gain from 29 to 49 days than control.

Zulkifli *et al.* (1993) observed that there was significant effect of feed restriction (60%) for 14 days on the body weight as by 43 days of age restricted chicks weighed less than those fed *ad libitum* regardless of genotype and sex. Senapati *et al.* (1997) reported that feed conversion efficiency of quails was significantly better after the restricted feeding period and concluded that restricted level of feeding exerts some sort of stress and slows normal growth, thereafter optimum feeding compensated for the poor growth and even enhanced the weight gain.

2. 5. 3. Other stressors

Reid *et al.* (1964) reported that cyclic high temperature stress brought a reduction in growth rate in broiler chicken with increased mortality and economic losses as a result of heat prostration when they attained the market age. Mortality resulting from heat prostration of broiler increases as the environmental temperature was increased to 38°C (Reece *et al.*, 1972). Donkoh (1989) observed that exposure of broiler chicken to the room temperature of 20°, 25°, 30° and 35°C there was a significant depression in growth rate, feed intake and efficiency of feed utilization with a significant increase in water consumption especially at the room temperature of 30°C and 35°C. However the mortality was not affected by environmental temperature.

Jaffar and Blaha (1996) and Sokolowicz *et al.* (1996) found that heat stress decreased the live weight gain and increased the mortality rate of broiler chicken. Guo *et al.* (1998) concluded that heat stress significantly reduced the feed intake, body weight gain, feed conversion efficiency and inhibited the normal development of lymphoid organs and impaired the immunological competence. Al-Batshan and Hussein (1999) observed that cyclic high temperatures reduced the body weight, feed intake, carcass weight and increased feed conversion ratio.

Puvadolpirod and Thaxton (2000d) reported that experimental induction of stress by continuous administration of ACTH(8 IU/kg/day intravenously for seven consecutive days) resulted in decreased body weight, relative weight of bursa, thymus, spleen and feed intakes. All physiological parameters with the exception of body weight and thymus weight returned to range of control values within one week after cessation of ACTH administration.

2.6 Antistress agents in poultry

2.6.1. L-Ascorbic acid

According to Roy and Guha (1958) and Chaudhuri and Chatterjee (1969) all poultry species are capable of synthesizing ascorbic acid in kidney. The quantity of ascorbic acid synthesized in avian species was supposed to be sufficient for normal growth and metabolism (Sealock and Goodland, 1951), but in stressful conditions the synthesized ascorbic acid was insufficient to meet the physiological needs (Lyle and Moreng, 1968; Freeman, 1971; Siegel, 1971; Hornig *et al.*, 1984). Ascorbic acid supplementation during stress resulted in a decreased synthesis of glucocorticoids in broiler chicken (Pardue and Thaxton, 1986).

Low mortality rate in birds fed with supplemental ascorbic acid during peak summer month was reported by Ahmad *et al.* (1967) and Herrick and Nockels (1969). Adding ascorbic acid to the diets of unstressed birds did not influence either the body weight or H:L ratio. This lack of response was also indicated by the results of Sifri *et al.* (1977) and Nickerek *et al.* 1989, and they concluded that if favorable husbandry conditions are provided, chicks will be able to synthesize their own ascorbic acid requirements. Bendich *et al.* (1984) reported that the immunoresponsiveness of T and B lymphocytes of guinea pigs were enhanced by diets containing higher than standard levels of vitamin C and E supplementation.

Addition of 1,000 mg/kg ascorbic acid reduced weight losses and mortality in birds up to four weeks of age following an acute heat exposure (Thaxton and Pardue, 1984). However, no improvement in weight gain or survivability of the birds was observed when ascorbic acid was provided in the diet at either 1000 mg/kg (Freeman *et al.*, 1983; Pardue *et al.*, 1985) or at 1 per cent (Brown and Southern, 1985). Degkwitz (1987) reported that ascorbic acid supplementation increased circulating thyroid hormones and lowered blood levels of cortisol in guinea pigs. The supplemented ascorbic acid had improved the performance in poultry subjected to disease (Gross, 1992) and heat stress (Kutlu and Forbes, 1993) and also alleviated stress induced increase in plasma corticosterone (Satterlee *et al.*, 1989) and H:L ratio (Pardue and Williams, 1990; Gross, 1992).

Gross (1988a) reported that as the stressfulness of the environment increased (measured by increasing H:L ratios), resistance of chicken to *Escherichia coli* infection was increased. At a relatively low level of environmental stress (H:L ratio = 0.33) the incidence of severe lesions was 22 per cent in chicken fed ascorbic acid @ 330 mg/kg and 80 per cent in control. He also reported that antibody titers were reduced when level of ascorbic acid was greater than 220 mg/kg against sheep erythrocytes injected intravenously.

Gross (1988b) reported that Leghorn type chicken in a relatively low stress environment was fed with diets containing 0-1100 mg of ascorbic acid per kg diet for a day before they were subjected to overheating by direct exposure to sun (29.1°C) and observed that as the dose of ascorbic acid increased from 0-330 mg/kg there was a decrease in mortality rate from 40 to 0 per cent. As the dose of ascorbic acid was increased further, there was an increase in mortality rate.

According to Satterlee *et al.* (1989) ascorbic acid supplementation reduced the stress response due to elevated plasma corticosterone level and H:L ratio which was evident in broiler chicken prepared for slaughter. Cheng *et al.* (1990) suggested that the ascorbic acid fed to hens in hot environment helped them to cope with the additional stresses of high bird density and humidity by dissipating more heat, providing an opportunity for increased feed intake and greater layer performance. According to them in laying hens under environmental stress supplemented with ascorbic acid had small influence on egg quality with a reduction in mortality rate. Johnston and Huang (1991) reported that ascorbic acid enhanced lymphocyte proliferation by improving the responsiveness of T-lymphocytes to mitogens.

Sahota *et al.* (1994) stated that ascorbic acid supplementation significantly increased the values of TEC, VPRC, Hb and decreased ESR of chicken exposed to heat stress. Abd-Ellah (1995) reported that addition of ascorbic acid at 250 mg and 500-mg/kg diet markedly reduced mortality (8%) compared with the control (28%). Ascorbic acid supplementation was able to neutralize the negative effect caused by heat stress. Ascorbic acid improved nutrient utilization leading to an increased production.

Ascorbic acid had been demonstrated to enhance antioxidant activity of alpha tocophorol by reducing the tocopheroxy radicals back to their active form of vitamin E (Jacob, 1995) or by sparing available vitamin E (Retsky and Frei, 1995). Dietary ascorbic acid supplementation improved body weight gain and feed conversion ratio and reduced the effects of heat stress (Kassim and Norziha, 1995). McKee and Harrison (1995) studied the effects of supplemental ascorbic acid on the performance of broiler chicken exposed to multiple concurrent stressors and found that ascorbic acid increased feed intake and lowered plasma corticosterone and H:L ratio.

Dzhambulatov *et al.* (1996) recommended the optimum vitamin C and vitamin B12 levels of 94 mg/kg and 35 μg/kg respectively in bulk feeds for broilers in order to prevent heat stress and increased productivity under hot climatic conditions of Southern Russian federation. Jaffar and Blaha (1996) reported that mortality was 10 per cent less in groups supplemented with ascorbic acid. They also found that electrolyte and vitamin supplementation increased live weight gain by 4.10 per cent with 1.5 per cent less feed intake than those receiving just vitamin supplement alone.

According to Sayed and Shoeib (1996) ascorbic acid supplementation in drinking water (0.5 g/lit) or diet (200 mg/kg diet) benefited heat stressed broilers and reduced mortality related to heat stress and improved body weight by 6.8 per cent.

McKee *et al.* (1997) reported that feed deprivation increased plasma hydroxybutyrate with no effect of dietary ascorbic acid and decreased plasma triglycerides in unsupplemented birds and plasma triglycerides remained elevated in birds supplemented with ascorbic acid. They concluded that ascorbic acid supplementation influenced body energy stores that are used for energy purposes during periods of reduced energy intake.

Mohammed (1997) stated that ascorbic acid supplementation (at the rate of 0, 150, 300 or 450 mg/kg feed) improved body weight of chicken, especially when pelleted diets were used. An interaction between level of ascorbic acid and diet form was observed for body weight at four, six and seven weeks of age and for feed conversion efficiency at four weeks of age. There was no difference in body weight of males and females in response to ascorbic acid supplementation. Elmoty *et al.* (1999) studied the effects of ascorbic acid administration during cold stress and found that ascorbic acid injection reduced mortality and improved body weight, feed conversion efficiency, levels of total protein, glucose, triiodothyronine and ascorbic acid in blood, gonads, kidneys, spleen and thyroid gland.

Zulkifli *et al.* (2000a) reported that in birds the H:L ratio was similar for ascorbic acid treated (1200 ppm) and control groups at 0 h, whereas 20 h after either upright or inverted handling treatment, the ascorbic acid supplemented birds had lower H:L ratio than control and reduced fearfulness. Al-Daraji *et al.* (2001) reported that supplementation of ascorbic acid in diets showed a highly significant decline in H:L ratio in the males and females as compared to control. Birds that received 450 ppm ascorbic acid had the lowest H:L ratio with a highly significant decline in the level of plasma glucose, aspartate amino transferase (AST) and alanine amino transferase (ALT), and significant increase in concentration of plasma protein, ascorbic acid, calcium, phosphorus and alkaline phosphatase (ALP) activity.

Puthpongsiripom *et al.* (2001) reported that hens supplemented with a combination of 65 IU alpha-tocopherol and 1000 ppm ascorbic acid per kg had an additive improvement in *in vitro* lymphocyte proliferation in response to Con A and LPS mitogens when compared with alpha tocopherol or ascorbic acid supplementation alone.

2.6.2. Herbal antistress agents (Zeetress®, Stresszee®)

Brekhman and Dardymov (1969) demonstrated that certain plant extracts possess anti-stress activity by inducing a state of non-specific increased resistance in animals and man. Antistress or adaptogenic activity had been reported in several plants used in the Chinese and other Southeastern Asian systems of Medicine, including *Panax ginseng, Eleutherococcus senticos, carlina biebersieini*, *Raponticum carthamoides*, *Rhodiola rosea* and *Gingko biloba* (Shibata *et al.*, 1985). In India antistress properties of *Withinia somnifera* (Bhattacharya *et al.*, 1987; Ghosal *et al.*, 1989), *Ocimum Sanctum*

(Bhargava and Singh, 1981 and Singh *et al.* 1978) and Shilajit (Ghosal *et al.* 1989 and Ghosal, 1992) had been documented.

Khansari *et al.* (1990) and Harbuz and Lightman (1992) reported that stressors cause immune dysfunction of an organism. According to Geetha (1993) stress induced hypercholestrolemia in control rats was significantly attenuated by Stresszee® (a product similar to Zeetress® for human use) at a dose of 10 mg/kg indicating antiatherogenic action of Stresszee® with a faster mobilization of glucose in stressed animals. It was also pointed out by Geetha (1993) and Kamath (1994) that stress induced changes in blood biochemical profiles of rats *viz.* increased glucose, urea, total protein as well as increased activity of different enzymes like AST, ALT, ALP and lactate dehydrogenase (LDH) in control animals were significantly decreased when treated with Stresszee®. Zeetress®, a polyherbal antistress adaptogen (contains the extracts of *Withinia somnifera* and *Ocimum sanctum* as major constituents) had been shown to ameliorate different undesirable effects of stress (Wheeler, 1993 and Das, 1994) and protected the animals from stress-induced alterations in ECG and blood pressure profile (Chatterjee, 1994a).

Agarwal (1994) reported that Stresszee® alleviated stress induced increase in plasma ACTH concentration in mice indicating that the mechanism of action of Stresszee in modulating the adrenocortical responses to stress. Bhattacharya and Ghosal (1994) reported that Zeetress® did not exert any discernible anti-stress activity on a single acute administration, but was shown to attenuate stress following subchronic administration (5-10 mg/kg orally) for seven days. Zeetress® reduced the incidence of stress induced gastric ulcerations and the level of plasma corticosterone, adrenal ascorbic acid and corticosterone and adrenal gland weight in rats . These findings provided an evidence for decrease in hypothalamic pituitary adrenocortical activity induced by stress.

Das and Chatterjee (1994) observed that administration of Stresszee® in rats resulted in an increase of lymphocyte population dynamics in differential leucocyte count with potentiated cellular and humoral components of immune system with consequent increase in the host defense against pathogenic stimuli. They also reported that Stresszee® was a safe product even at ten times higher dose level (100 mg/kg body weight) on daily administration for 30 days. The recommended oral therapeutic dose of Stresszee® being 10 mg/kg body weight. Stresszee® at dose rate of 30 and 100 mg/kg body weight produced an increase in bodyweight of rats and better-feed conversion ratio. There was a significant increase in lymphocyte count and plasma globulin value upon administration of Stresszee® at the dose rate of 30 mg/kg body weight. There was significant reduction in weight of adrenal gland and eosinophilia was detected when Stresszee® was administered at the dose of 100 mg/kg body weight.

Wheeler (1994) reported that Zeetress® treated broiler birds exhibited increase in body weight gain (15.83%) and improvement in feed conversion ratio (5.4%) and a reduction in mortality (45.83%) in comparison to untreated control birds subjected to similar stress in United Kingdom conditions. Malti *et al.* (1995) reported that supplementation of Zeetress® in drinking water @ 1 g/100 birds daily for 10 days improved the egg production and egg quality with little difference in feed consumption. Nadig *et al.* (2002) reported that Stenot® (a product with similar composition of Zeetress®) reduced the plasma corticosterone concentration in overcrowded rats. Literature about the dietary supplementation of Zeetress® in broiler chicken is meagre.

Materials and Methods

3. MATERIALS AND METHDOS

The experiment was conducted in Vencob strain of broiler chicken in the Department of Physiology, College of Veterinary and Animal Sciences, Mannuthy with the objective of evaluating the effects of induced stress and antistress agents on certain physiological and biochemical parameters, for a period of four weeks from fourth to eighth week of age.

3.1 Experimental rations

The standard broiler ration (starter and finisher) formulated as per Bureau of Indian Standards specifications (BIS, 1992) and proximate principle was estimated as per Association of Official Analytical Chemists (AOAC, 1990). Day old chicks were fed with broiler starter ration upto four weeks of age and with broiler finisher ration upto eight weeks of age. The ingredient composition and chemical composition of the above rations are presented in table 2 and 3 respectively.

3.2 Experimental birds

Fifty six, one day old broiler chicken (Vencob) procured from a commercial hatchery were reared in battery cages under standard managemental conditions upto four weeks of age. The chicks were selected randomly, weighed, wing banded and divided into seven groups comprising of eight birds in each group (table 1).

Table 1. Experimental groups of broiler chicken

Groups	Stressor	Antistress agent	Remarks
G-I	Nil	Nil	Control (floor space 0.79 sqft/bird or 734 sq.cm/bird).
G-II	Overcrowding	Nil	Floor space reduced by 33 per cent (0.529 sq.ft/bird or 492 sq.cm/bird).
G-III	Overcrowding	Zeetress® *	Floor space (0.529 sq.ft/bird) and supplemented with Zeetress® @ 0.01 per cent in diet
G-IV	Overcrowding	Ascorbic acid**	Floor space (0.529 sq.ft/bird) and supplemented with ascorbic acid @ 0.02 per cent in diet
G-V	Feed restriction	Nil	30 per cent feed restriction
G-VI	Feed restriction	Zeetress® *	30 per cent feed restriction and supplemented with Zeetress® @ 0.01 per cent in diet
G-VII	Feed restriction	Ascorbic acid**	30 per cent feed restriction and supplemented with ascorbic acid @ 0.02% in diet.

* Zeetress® water soluble powder - M/s. Natural Remedies Pvt. Ltd., Bangalore. Each 10 g contains extracts of Gramya 3g, Varahkarni 3g, Virishya 3g and Base 1g.

** L-Ascorbic Acid (vitamin C) powder - M/s. Sisco Research Laboratories Pvt. Ltd., Mumbai.

Birds of groups G-I, G-II, G-III and G-IV were fed *ad libitum* whereas feed restriction (30%) was imposed for birds of groups G-V, G-VI and G-VII. They were fed twice daily (morning and evening). The quantity of daily feed allowance for restricted group was decided based on the previous days feed intake of birds *ad libitum* fed (control) group i.e. by providing 70% of the feed consumed by control birds (Zulkifli *et al.*, 2000b). Birds were provided with clean drinking water *ad libitum* during the entire experimental period.

Feed intake of birds were recorded replication wise at the end of each week and the average daily feed consumption per bird was calculated. The body weight of individual birds were recorded at weekly intervals from fourth week to eighth week of age.

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

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

3.3 Blood collection

Blood samples (6 ml) were collected from wing vein with anticoagulant (EDTA 2 mg/ml blood) initially at fourth week of age and at fortnight intervals (at sixth and eighth week of age). Part of the blood was used for estimation of haematological parameters and the remaining blood was centrifuged at 3000 rpm for 30 min to separate the plasma. One ml of blood was collected using sodium fluoride as anticoagulant (10 mg/ml blood) for plasma glucose

Table 2. Composition of broiler chicken ration

Sl. No.	Ingredients	Standard broiler ration (kg)	
		Starter	Finisher
1.	Yellow maize	44.00	54.00
2.	Ground nut cake	27.00	19.00
3.	Soyabean meal	6.00	7.00
4.	Gingelly oil cake	3.00	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	100.00	100.00
Added per 100 kg of feed			
9.	Vitamin mixture (g)**	15.00	15.00
10.	Lysine hydrochloride (g)	200.00	200.00
11.	Methionine (g)	100.00	Nil
12.	Coccidiostat (g)***	50.00	50.00
13.	Manganese sulphate (g)	10.00	10.00

* Keyes Mineral mixture®(M/s Kerala Solvent Extractions Limited, Irinijalakuda, Kerala, India):

Calcium 32%, Phosphorus 6%, Magnesium 1000 ppm, Cobalt 60 ppm, Zinc 2600 ppm, Iron 0.1%, Iodine 100 ppm, Copper 100 ppm and Manganese 2700 ppm.

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

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

Table 3. Proximate principle of broiler chicken ration (on dry matter basis)

Sl. No.	Nutrients	Standard broiler ration (%)	
		Starter	Finisher
Analysed values*			
1.	Moisture	9.60	9.48
2.	Crude protein	23.54	20.35
3.	Ether extract	5.87	5.95
4.	Crude fibre	5.28	4.96
5.	Nitrogen free extract	54.01	57.32
6.	Total ash	11.30	11.42
7.	Acid insoluble ash	2.46	2.50
8.	Calcium	1.40	1.34
9.	Phosphorus	0.80	0.73
Calculated values			
10.	Metabolizable energy (kcal/kg)	2802.00	2904.00
11.	Lysine (%)	1.50	1.00
12.	Methionine (%)	0.53	0.40
13.	Manganese (mg/kg)	104.00	102.00

* Mean of eight samples

estimation. One ml of blood was collected with heparin as anticoagulant (20 IU/ml blood) for mitogen induced lymphocyte proliferation test. Plasma samples were aliquoted and stored at –20°C for hormonal estimation and at –4°C for biochemical analysis.

3.4 Haematological parameters

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

Blood smears were prepared on a clean grease free slide using fresh blood. Air dried smears were stained using Wright-Giemsa stain solution as described by Shen (1983) and different leucocytes were counted and their percentages were calculated. Heterophil: Lymphocyte (H:L) ratio was determined by dividing the percent heterophils by percent lymphocytes (Gross and Siegel, 1983).

Mitogen induced lymphocyte blastogenic response (semi-quantitative method) was conducted by the method suggested originally by Talwar (1983) and modified by Shibu *et al.* (2001).

Principle:

The mitogen (phytohaemagglutinin) induced lymphocyte blastogenic response in culture vary under different concentrations of plasma corticosteroids.

Reagents used:

1. RPMI 1640 (Hi-Media Laboratories Ltd., Mumbai): Dried tissue culture medium was rehydrated to one litre using double distilled water (DDW) and pH adjusted to 7.2 and filtered through 0.22 µm cellulose acetate filter aseptically.
2. Bacto-Phytohaemagglutinin-M-1% (PHA-M) (Difco Laboratories, Detroit, Michigan, USA): freeze dried powder reconstituted with double distilled water (DDW).
3. Ficol-Paque solution (SISCO Research Laboratories Pvt. Ltd., Mumbai) for density gradient centrifugation.
4. Seven lakh U of penicillin (M/s Alembic Ltd., Vadodara) and 700 mg of streptomycin (M/s Sarabhai Piramal Pharmaceuticals Ltd., Vadodara) were incorporated per litre of medium.

5. Phosphate buffered saline (PBS-pH 7.2)

A. 1.7 g of KH_2PO_4 was dissolved in 250 ml DDW

B. 4.45 g of $Na_2HPO_4 . 2H_2O$ was dissolved in 500 ml DDW

C. 250 ml of A was mixed with 387.5 ml of B to prepare PBS and the pH was adjusted to 7.2

Procedure:

Three ml of heparinised blood was diluted two times with phosphate buffered saline (PBS - pH 7.2) and carefully layered over 3 ml of ficol-paque solution in a centrifuge tube and centrifuged at 3000 rpm for 20 min. Lymphocytes at the ficol-plasma interface were aspirated with a Pasteur pipette and washed thrice with PBS in order to remove traces of ficol-paque and resuspended in 10 ml of PBS.

Ten microlitre of trypan blue (1%) was added to 100 µl of resuspended solution to find out the percentage livability of lymphocyte by dye exclusion. The actual live lymphocytes number in resuspended solution was determined haemocytometrically using WBC chamber. Then the dilution was adjusted so as to get 1 million live lymphocytes per ml.

Seven ml of sterile RPMI-1640 medium was taken in a culture vial into which 150 µl PHA-M and 500 µl of solution containing 0.5 million of lymphocytes were added. The final volume was made upto 10 ml in triplicates using pooled sterile plasma separated from birds of the study. Incubation was carried out for 72 h at 37°C. At the end of 72 h, lymphocyte number was determined haemocytometrically and the results were expressed as number of lymphocytes present per 500 μl. The mitogenic response of lymphocyte number was compared with the control.

3.5 Biochemical parameters

3.5.1 Plasma proteins

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

The plasma globulin content was determined by substracting plasma albumin level from total plasma protein content and subsequently A:G ratio was calculated.

3.5.2 Plasma lipid profile

Concentration of plasma total lipids was estimated by Phosphovainilline method as described by Chabrol (1961) using Labkit® kit (M/s. Labkit; Spain).

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

The total cholesterol level was estimated by Cholesterol phenol amino antipyrine (CHOD-PAP) method, as suggested by Richmond (1973) using Ecoline® kit (M/s. E. Merck (India) limited, Mumbai).

3.5.3 Plasma glucose

Plasma glucose was estimated by Glucose oxidase-peroxidase (GOD-POD) method as described by Mayne (1994) using Ecoline® kit. (M/s. E. Merck (India) Limited, Mumbai).

3.5.4 Plasma uric acid

Plasma uric acid concentration was estimated by the method described by Trinder (1969) using Ecoline® kit (M/s. E. Merck (India) limited, Mumbai).

3.6 Hormonal assay

Concentration of plasma cortisol was estimated by radio-immuno-assay (RIA) technique using the gamma coat cortisol radio immunoassay commercial kit (M/s. Diasorin, Minnesota, U.S.A).

3.7 Statistical analysis

Data collected on various parameters were statistically analyzed by employing ANOVA and Dunkan's multiple range test (DMRT) for comparing different groups and periods as per the methods described by Snedecor and Cochran (1989).

Results

4. RESULTS

4.1. Effect of induced stress and antistress agents on haematological parameters

The values of certain haematological parameters such as total erythrocyte count (TEC), haemoglobin (Hb) concentration, total leucocyte count (TLC), volume of packed red cells (VPRC), erythrocyte sedimentation rate (ESR), erythrocyte indices viz., mean corpuscular volume(MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) differential leucocyte count (DLC) and heterophil:lymphocyte (H:L) ratio of broiler chicken at fourth, sixth and eighth week of age (from fourth week to eighth week of age) under the effect of induced stress and antistress agents are depicted in tables 4a,4b,4c,4d, 4e and 4f.

4.1.1 Total erythrocyte count (TEC)

There was no significant difference (P>0.05) in TEC among various groups at fourth week of age (initial period) with values ranging from 2.933 ± 0.09 x 10^6/µl in birds of G-VI group (feed restriction) with dietary supplementation of Zeetress® to 3.045 ± 0.09 x 10^6/µl in birds of G-VII group (feed restriction) with dietary supplementation of ascorbic acid.

At sixth week of age there was no significant difference in TEC between birds of G-I group (control) and floor space reduced groups (G-II, G-III and

G-IV) whereas significantly ($P<0.05$) lower TEC values were recorded for feed restricted groups (G-V, G-VI and G-VII). The value of G-I (control) was 3.093 ± 0.03 x $10^6/\mu l$ whereas, birds of G-II group (floor space reduction without any dietary antistress agent) had the highest erythrocyte count of 3.117 ± 0.04 x $10^6/\mu l$. The values of G-III and G-IV groups were 3.113 ± 0.05 x $10^6/\mu l$ and 3.071 ± 0.04 x $10^6/\mu l$ respectively. At sixth week of age there was no significant influence ($P<0.05$) of Zeestress® and ascorbic acid supplementation on TEC in birds of G-VI and G-VII groups respectively when compared to G-V group. The birds of G-VI group had the highest value of 2.785 ± 0.05 x $10^6/\mu l$ and ascorbic acid supplemented group (G-VII) had mean value of 2.766 ± 0.04 x $10^6/\mu l$ and lowest value (2.729 ± 0.06 x $10^6/\mu l$) was observed in birds of G-V group.

At eighth week of age the birds of different groups showed a similar pattern in TEC as observed at sixth week of age. The birds of feed restricted groups (G-V, G-VI and G-VII) had significantly ($P<0.05$) lower TEC (2.631 ± 0.05 x $10^6/\mu l$, 2.699 ± 0.07 x $10^6/\mu l$ and 2.720 ± 0.06 x $10^6/\mu l$ respectively) when compared to either TEC in birds of control group G-I (2.935 ± 0.04 x $10^6/\mu l$ or of floor space reduced groups G-II, G-III and G-IV (2.990 ± 0.06 x $10^6/\mu l$, 3.031 ± 0.07 x $10^6/\mu l$ and 3.051 ± 0.05 x $10^6/\mu l$ respectively). The dietary supplementation of antistress agents (Zeetress and ascorbic acid) had no significant influence on TEC value either among the birds of floor space reduced groups (G-III and G-IV) or in feed restricted groups (G-VI and G-VII).

The birds of G-I group when compared among period had significantly ($P<0.05$) lower TEC ($2.935 \pm 0.04 \times 10^6/\mu l$) at eighth week of age when compared to TEC at sixth week of age ($3.093 \pm 0.03 \times 10^6/\mu l$). There was no significant influence ($P>0.05$) of age on TEC in birds of floor space reduced groups (G-II, G-III and G-IV) whereas in birds of feed restricted groups (G-V,G-VI and G-VII) a significantly ($P<0.05$) higher TEC was recorded at fourth week of age as given in table 4a. The TEC in birds of control (G-I) and floor space reduced groups (G-II, G-III and G-IV) showed a fluctuating trend in TEC with peak value at sixth week of age. A decreasing trend with age was recorded in TEC of the feed restricted groups (G-V, G-VI and G-VII).

4.1.2 Haemoglobin (Hb)

At fourth week of age, Hb values did not differ significantly ($P>0.05$) among various groups with value ranging from 9.94 ± 0.58 g% in birds of G-I group (control) to 10.38 ± 0.58 g% in birds of G-V group (feed restriction without any dietary antistress agent).

At sixth week of age the birds of feed restricted groups (G-V, G-VI and G-VII) had significantly ($P<0.05$) lower Hb values (8.81 ± 0.15 g%, 8.45 ± 0.18 g%, 8.67 ± 0.18 g% respectively.) as compared to those of control group G-I (10.63 ± 0.27g%) or floor space reduced groups (G-II 10.60 ± 0.21 g%, G-III 10.63 ± 0.44 g% and G-IV 10.34 ± 0.46 g%). There was no significant influence of Zeetress® and ascorbic acid supplementation on Hb value either in birds of floor space reduced group (G-III and G-IV respectively) or in feed restricted group (G-VI and G-VII group) at sixth week of age.

The Hb values at eighth week showed same pattern as that of sixth week of age (Table 4a) with control (G-I) value of 9.88 ± 0.22 g%. In birds of floor space reduced groups the value ranged from 9.88 ± 0.53 g% in G-III to 10.31 ± 0.28 g% in G-II. Among feed restricted groups the Hb value was highest in birds of G-VI group (8.79 ± 0.25 g%), and lowest Hb value was observed in G-V group (8.19 ± 0.22 g%)

The Haemoglobin value showed a fluctuating trend with age. There was no significant influence of age among Hb value in birds of control group (G-I) and floor space reduced groups (G-III and G-IV) with the peak Hb concentration at sixth week of age. However, the birds of feed restricted groups (G-V, G-VI and G-VII) showed a significantly ($P<0.05$) higher Hb value at fourth week of age. In birds of G-II group, the sixth week Hb value was significantly ($P<0.05$) higher as compared to Hb value of fourth or eighth week of age (table 4a).

4.1.3. Volume of packed red cells (VPRC)

At fourth week of age there was no significant difference in VPRC (as per microhaematocrit method) value among various groups (Table 4a). The value ranged between 27.88 ± 1.13% in birds of G-III group (floor space reduced) with dietary supplementation of Zeetress® and 30.38 ± 1.34 % in birds of G-VII group (feed restricted and supplemented with ascorbic acid.

At sixth week of age the birds of feed restricted groups (G-V, G-VI and G-VII) showed significantly ($P<0.05$) lower VPRC when compared to control group (G-I). The VPRC was highest in birds of control group G-I (31.63 ± 0.73%) and lowest for feed restricted group (G-V) without any dietary antistress agent (26.38 ± 0.37%). There was no significant variation ($P>0.05$) in VPRC value of control (G-I) and floor space reduced group (G-II). Birds of floor space reduced (G-III and G-IV) and feed restricted groups (G-VI and G-VII) had no significant influence of dietary supplementation of either Zeetress® or ascorbic acid, but higher VPRC value was observed in birds supplemented with Zeetress® (G-III and G-VI).

At eighth week of age the birds of feed restricted groups (G-V, G-VI, G-VII) had significantly ($P<0.05$) lower VPRC value when compared to either control (G-I) or floor space reduction stress groups (G-II, G-III and G-IV). At eighth week of age the control group (G-I) had VPRC of 29.63 ± 0.60 %. There was no significant difference in VPRC of birds in control (G-I) or floor space reduced groups (G-II, G-III and G-IV). Dietary supplementation of Zeetress® or ascorbic acid had no significant influence on the VPRC value of birds in floor space reduced groups (G-III and G-IV) but in birds of feed restricted group G-VII a significantly ($P<0.05$) lower VPRC value was observed when compared that of G-V.

There was no significant influence of age on VPRC value in birds of groups G-I, G-II, G-III and G-IV (Table 4a) but the value being highest at sixth week of age. In G-V, G-VI and G-VII the sixth week and eighth week VPRC value was significantly ($P<0.05$) lower when compared to fourth week value and showed a decreasing trend (Table 4a).

4.1.4. Erythrocyte sedimentation rate (ESR)

The ESR value did not vary significantly among various experimental groups at fourth week of age (Table 4b). The ESR value ranged between 1.625 ± 0.26 mm/h for G-IV and 2.125 ± 0.31 mm/h for birds of G-I group.

At sixth week of age the value there was no significant ($P>0.05$) variation in ESR value among various experimental groups. The value ranged between 1.875 ± 0.29 mm/hr in birds of G-V group to 2.625 ± 0.18 mm/h in birds of G-III and G-IV group.

At eighth week of age, the ESR in birds of G-V group (feed restricted without any antistress agent) was found to be significantly ($P<0.05$) lower (1.750 ± 0.16 mm/h) when compared to G-I group (2.625 ± 0.18 mm/h) as well as birds of the other groups G-II, G-III, G-IV, G-VI and G-VII. There was no significant variation in ESR value of control group (G-I), floor space reduced groups (G-II 2.625 ± 0.18 mm/h, G-III 2.375 ± 0.18 mm/h and G-IV 2.500 ± 0.19 mm/h) and feed restricted groups (G-VI and G-VII) as given in table 4b. The dietary supplementation of Zeetress or ascorbic acid failed to show any significant influence on ESR value of both feed restricted (G-VI and G-VII) and floor space reduced groups (G-III and G-IV) (table 4b).

There was no significant influence of age on ESR values of birds in control (G-I) and feed restricted groups (G-V, G-VI and G-VII). In floor space reduced groups (G-II, G-III and G-IV) the ESR value was found to be significantly ($P<0.05$) lower at fourth week of age (table 4b).

The erythrocyte sedimentation rate (ESR) showed a fluctuating trend between periods in floor space reduced groups G-III and G-IV. An increasing trend was observed in birds of control group G-I, floor space reduced group G-II and in feed restricted groups G-V and G-VI whereas in feed restricted group G-VII with dietary supplementation of ascorbic acid, the fourth and sixth week value was same (2.000 ± 0.27 mm/h) with eighth week ESR value being highest (Table 4b).

4.1.5. Total leucocyte count (TLC)

At fourth week of age, there was no significant variation ($P>0.05$) in total leucocyte count (TLC) of broiler chicken among control (G-I) and floor space reduced groups (G-II, G-III and G-IV) as given in table 4b. The birds of feed restricted group G-VII with dietary supplementation of ascorbic acid showed a significantly ($P<0.05$) lower TLC (23.86 ± 0.94 thousand/μl) when compared to control group G-I (26.49 ± 0.83 thousand/μl). There was no significant variation in TLC among the birds of floor space reduced groups G-II, G-III and G-IV (26.24 ± 0.93 thousand/μl, 25.06 ± 0.81 thousand/μl, 26.39 ± 0.90 thousand/μl respectively) and feed restricted groups G-V and G-VI (24.55 ± 0.63 thousand/μl and 25.84 ± 1.10 thousand/μl).

At sixth week of age the birds of G-V group (feed restricted without any antistress agent) had significantly ($P<0.05$) lower TLC value of 23.46 ± 0.69

thousand/μl compared to G-I group (control). There was no significant variation in TLC values in birds of control (G-I) group and space reduced groups (G-II, G-III and G-IV). The dietary Zeetress® and ascorbic acid supplementation failed to show any significant influence on TLC either in floor space reduced groups (G-III and G-IV) or in feed restricted groups (G-VI and G-VII) when compared to G-II and G-V groups respectively.

At eighth week of age the birds in feed restricted group (G-V, G-VI and G-VII) had significantly ($P<0.05$) lower TLC (19.66 ± 0.74 thousand/μl, 20.75 ± 0.84 thousand/μl and 20.85 ± 0.78 thousand/μl respectively) when compared to that of control group (G-I). There was no significant difference in TLC of control group G-I and floor space reduced groups G-II, G-III and G-IV. The highest value (23.74 ± 0.82 thousand/μl) was observed in birds of G-I group (control) and floor space reduced groups G-II, G-III and G-IV had a TLC of 23.58 ± 0.79 thousand/μl, 23.13 ± 0.65 thousand/μl and 23.30 ± 0.93 thousand/μl respectively.

Zeetress® and ascorbic acid supplementation failed to show any significant influence ($P>0.05$) on TLC value in birds of both feed restricted groups (G-VI and G-VII respectively) and the floor space reduction stress groups (G-III, G-IV respectively).

Among the periods, when compared to G-V and G-II group respectively all the groups (G-I to G-VII) had a significantly ($P<0.05$) lower TLC value at eighth week of age than that of the fourth and sixth week of age (Table 4b.)

Table 4a. Effect of induced stress and antistress agents on haematological parameters of broiler chicken from fourth to eighth week of age, Mean ± SE (n=8)

PARAMETER	TEC (millions/µl)			Hb (g%)			VPRC (%)		
Periods / Groups	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week
G – I (Control)	3.042^{axy} ± 0.06	3.093^{ax} ± 0.03	2.935^{ay} ± 0.04	9.94^{ax} ± 0.58	10.63^{ax} ± 0.27	9.88^{ax} ± 0.22	29.75^{ax} ± 1.13	31.63^{ax} ± 0.73	29.63^{ax} ± 0.60
G – II (Floor space reduction)	3.025^{ax} ± 0.08	3.117^{ax} ± 0.04	2.990^{ax} ± 0.06	10.26^{ay} ± 0.51	10.60^{ax} ± 0.21	10.31^{ay} ± 0.28	29.62^{ax} ± 1.13	30.75^{ax} ± 0.37	30.25^{ax} ± 0.65
G – III (Floor space reduction + Zeetress)	3.018^{ax} ± 0.08	3.113^{ax} ± 0.05	3.031^{ax} ± 0.07	9.68^{ax} ± 0.45	10.63^{ax} ± 0.44	9.88^{ax} ± 0.53	27.88^{ax} ± 1.13	31.00^{ax} ± 0.93	30.25^{ax} ± 1.35
G – IV (Floor space reduction + Ascorbic acid)	3.022^{ax} ± 0.08	3.071^{ax} ± 0.04	3.051^{ax} ± 0.05	10.10^{ax} ± 0.41	10.34^{ax} ± 0.46	10.10^{ax} ± 0.47	28.64^{ax} ± 1.10	30.88^{ax} ± 0.79	30.00^{ax} ± 1.18
G – V (Feed restriction)	3.011^{ax} ± 0.07	2.729^{by} ± 0.06	2.631^{by} ± 0.05	10.38^{ax} ± 0.58	8.81^{by} ± 0.15	8.19^{by} ± 0.22	29.38^{ax} ± 1.38	26.38^{by} ± 0.37	25.50^{by} ± 0.91
G – VI (Feed restriction + Zeetress)	2.933^{ax} ± 0.09	2.785^{by} ± 0.05	2.699^{by} ± 0.07	10.05^{ax} ± 0.55	8.45^{by} ± 0.18	8.79^{by} ± 0.25	28.78^{ax} ± 1.35	28.13^{bx} ± 0.67	26.50^{bx} ± 0.71
G – VII (Feed restriction + Ascorbic acid)	3.045^{ax} ± 0.09	2.766^{by} ± 0.04	2.720^{by} ± 0.06	10.28^{ax} ± 0.53	8.67^{by} ± 0.18	8.65^{by} ± 0.23	30.38^{ax} ± 1.34	23.88^{cy} ± 0.87	26.00^{by} ± 0.71

Mean ± SE (between groups) bearing different superscripts (a, b, c) in columns differ significantly ($P<0.05$)
Mean ± SE (between periods) bearing different superscripts (x, y) in rows for each parameter differ significantly ($P<0.05$)

Table 4b. Effect of induced stress and antistress agents on haematological parameters of broiler chicken from fourth to eighth week of age, Mean ± SE (n=8)

PARAMETER / Periods / Groups	ESR (mm/h)			TLC (thousand/µl)		
	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week
G – I (Control)	2.125^{ax} ± 0.31	2.250^{ax} ± 0.31	2.625^{ax} ± 0.18	26.49^{ax} ± 0.83	26.33^{ax} ± 0.40	23.74^{ay} ± 0.82
G – II (Floor space reduction)	1.875^{ay} ± 0.29	$2.500^{a\,xy}$ ± 0.19	2.625^{ax} ± 0.18	26.24^{abx} ± 0.93	26.01^{abx} ± 0.72	23.58^{ay} ± 0.79
G – III (Floor space reduction + Zeetress)	1.675^{ay} ± 0.23	2.625^{ax} ± 0.18	2.375^{axy} ± 0.18	25.06^{abxy} ± 0.81	25.81^{abx} ± 0.76	23.13^{aby} ± 0.65
G – IV (Floor space reduction + Ascorbic acid)	1.625^{ay} ± 0.26	2.625^{ax} ± 0.18	2.500^{ax} ± 0.19	26.39^{ax} ± 0.90	26.31^{ax} ± 0.57	23.30^{ay} ± 0.93
G – V (Feed restriction)	1.875^{ax} ± 0.23	1.875^{ax} ± 0.29	1.750^{bx} ± 0.16	24.55^{abx} ± 0.63	23.46^{cx} ± 0.69	19.66^{cy} ± 0.74
G – VI (Feed restriction + Zeetress)	1.750^{ax} ± 0.25	2.000^{ax} ± 0.27	2.250^{abx} ± 0.16	25.84^{abx} ± 1.10	24.23^{bcx} ± 0.58	20.75^{bcy} ± 0.84
G – VII (Feed restriction + Ascorbic acid)	2.000^{ax} ± 0.27	2.000^{ax} ± 0.27	2.125^{abx} ± 0.23	23.86^{bx} ± 0.94	24.34^{bcx} ± 0.58	20.85^{cy} ± 0.78

Mean ± SE (between groups) bearing different superscripts (a, b, c) in columns differ significantly ($P<0.05$)
Mean ± SE (between periods) bearing different superscripts (x, y) in rows for each parameter differ significantly ($P<0.05$)

4.1.6. Erythrocyte indices

4.1.6.1 Mean corpuscular volume (MCV).

At fourth week of age there was no significant difference (P>0.05) in MCV value within groups and the values ranged between 95.468 ± 1.27 fl (femto liter) in birds of G-III group (floor space reduction) with dietary supplementation of Zeetress®) and 99.481 ± 1.73 fl in birds of G-VII (feed restricted) group with dietary supplementation of ascorbic acid (table 4c).

At sixth week of age the MCV in birds of G-VII feed restricted group with dietary supplementation of ascorbic acid was significantly (P<0.05) lower (86.554 ± 3.83 fl) when compared to control group (G-I) and other treatment groups. In birds of G-II, G-III, G-IV, G-V and G-VI treatment groups values ranged between 96.882 ± 1.88 fl in G-V group (feed restricted without any antistress agent) to 102.294 ± 2.28 fl in birds of control group G-I. There was no significant difference in MCV value of birds in G-I group (control) with that of floor space reduced groups G-II, G-III and G-IV and feed restricted groups G-V and G-VI. There was no significant influence (P>0.05) of dietary Zeetress® and ascorbic acid supplementation on MCV value in birds of floor space reduced groups (G-III and G-IV) whereas dietary ascorbic acid supplementation in feed restricted group caused significantly (P<0.05) lower MCV value when compared to G-V group without any dietary antistress agent.

At eighth week of age there was no significant (P>0.05) variation in MCV within groups and the highest MCV was recorded in birds of G-II group

(101.209 ± 1.52 fl) and lowest MCV in birds of G-VII group (95.650 ± 1.43 fl). Dietary supplementation of Zeetress® (G-III and G-VI) or ascorbic acid had no significant influence ($P>0.05$) on MCV values of both (G-III and G-IV) and feed restricted groups (G-VI and G-VII) at eighth week of age (table 4c).

Among the periods a significantly ($P<0.05$) lower MCV value was recorded at sixth week of age in birds of G-VII (feed restricted group with dietary supplementation of ascorbic acid). In birds of feed restricted group G-VI with dietary supplementation of Zeetress the fourth week MCV value was significantly ($P<0.05$) lower. In birds of other treatment groups (G-I, G-II, G-III, G-IV and G-V) there was no significant influence of age on MCV values (table 4c).

4.1.6.2 Mean corpuscular haemoglobin (MCH)

At fourth week of age there was no significant difference in MCH value among various experimental groups. The birds of feed restricted group without any dietary antistress agent (G-V) had the highest value (34.286 ± 1.13 pg) and in birds of floor space reduced group with dietary supplementation of Zeetress® (G-III) had the lowest value (31.984± 0.67 pg).

At sixth weeks of age in the birds of control group (G-I) and floor space reduced groups (G-II, G-III and G-IV) there was no significant difference in MCH value whereas in birds of feed restricted groups (G-VI and G-VII) with dietary antistress agent (30.335 ± 0.33 pg and 31.349 ± 0.36 pg respectively) had significantly (P<0.05) lower MCH value was observed when compared to control group G-I (34.655 ± 0.84 pg) (Table 4c).

At eighth week of age there was no significant difference in MCH values among the birds of floor space reduced groups (G-II, G-III and G-IV) and that of control group (G-I). The feed restricted group G-V without antistress agent in diet, had significantly (P<0.05) lowest (31.093 ± 0.39 pg) MCH value when compared to that of control group (G-I) and other experimental groups. Dietary supplementation of Zeetress® or ascorbic acid had no significant influence on MCH values in either floor space reduced (G-III and G-IV) or feed restricted groups (G-VI and G-VII) both at sixth and eighth week of age (table 4c).

There was no significant influence of age on MCH value in birds of control group (G-I) and floor space reduced groups (G-II, G-III and G-IV). In birds of feed restricted groups (G-V, G-VI and G-VII) significant variation was there between fourth, sixth and eighth week MCH value was recorded as given in table 4c.

4.1.6.3. Mean corpuscular haemoglobin concentration (MCHC).

At fourth week of age there was no significant difference (P<0.05) in MCHC value of birds in G-II (floor space reduction without any dietary antistress agent) and control group G-I (33.285 ± 1.03 g%) whereas a significantly lower (P<0.05) MCHC value was recorded in birds of control (G-I) group when compared to birds of G-V group (feed restricted) without any dietary antistress agent) (35.133 ± 0.45 g%), and in birds of all other treatment groups (G-II, G-III, G-IV, G-VI and G-VII) there was no significant variation in MCHC value (Table 4c).

At sixth week of age there was no significant difference in MCHC value of G-II group and that of control (G-I) group, and also there was no significant difference in MCHC value of G-V group and that of control (G-I) group. Dietary Zeetress® and ascorbic acid supplementation had no significant influence on MCHC values in floor space reduced groups (G-II and G-IV), whereas in feed restricted groups, Zeetress® supplementation (G-VI) resulted in significantly ($P<0.05$) lower and ascorbic acid supplementation (G-VII) resulted in significantly ($P<0.05$) higher MCHC values when compared to their respective groups G-II and G-V (table 4c).

At eight week age there was no significant variation in MCHC value in birds of G-II group (floor space reduced group) without any dietary antistress agent) and control (G-I) group, and also no significant variation MCHC value was recorded and in birds of G-V group (feed restricted group without any antistress agent) and control (G-I) group. The birds of feed restricted group G-V had the lowest (32.189 ± 0.45 g%) MCHC. Dietary Zeetress® and ascorbic acid supplementation failed to show any significant influence on MCHC values either in birds of floor space reduced groups (G-III and G-IV) or feed restricted groups (G-VI and G-VII respectively) at eighth week of age.

Table 4c. Effect of induced stress and antistress agents on erythrocyte indices of broiler chicken from fourth to eighth week of age, Mean ± SE (n=8)

PARAMETER	MCV (fl)			MCH (pg)			MCHC (%)		
Periods / Groups	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week
G – I (Control)	97.619^{ax} ± 2.09	102.294^{ax} ± 2.28	100.93^{ax} ± 0.71	32.557^{ax} ± 1.42	34.655^{ax} ± 0.84	33.655^{abx} ± 0.63	33.285^{bx} ± 1.03	33.592^{bx} ± 0.28	33.344^{abx} ± 0.43
G – II (Floor space reduction)	97.872^{ax} ± 2.42	98.675^{ax} ± 1.15	101.209^{ax} ± 1.52	33.818^{ax} ± 0.92	33.997^{ax} ± 0.51	34.478^{ax} ± 0.62	34.575^{abx} ± 0.61	34.453^{abx} ± 0.31	34.065^{ax} ± 0.31
G – III (Floor space reduction + Zeetress)	95.468^{ax} ± 1.27	99.474^{ax} ± 1.52	96.185^{ax} ± 2.46	31.984^{ax} ± 0.67	34.049^{ax} ± 0.87	32.474^{abcx} ± 1.21	33.483^{abx} ± 0.33	34.201^{abx} ± 0.45	33.757^{ax} ± 0.82
G – IV (Floor space reduction + Ascorbic acid)	97.844^{ax} ± 1.48	100.485^{ax} ± 1.92	98.239^{ax} ± 3.08	33.336^{ax} ± 0.55	33.706^{ax} ± 1.12	33.048^{abcx} ± 1.21	34.087^{abx} ± 0.46	33.541^{bx} ± 0.91	33.624^{abx} ± 0.45
G – V (Feed restriction)	97.256^{ax} ± 2.63	96.882^{ax} ± 1.88	96.791^{ax} ± 2.26	34.286^{ax} ± 1.13	32.380^{abxy} ± 0.77	31.093^{cy} ± 0.39	35.133^{ax} ± 0.45	33.440^{by} ± 0.60	32.189^{by} ± 0.45
G – VI (Feed restriction + Zeetress)	95.750^{ay} ± 1.86	100.915^{ax} ± 1.00	98.212^{axy} ± 1.12	33.405^{ax} ± 0.91	30.335^{by} ± 0.33	32.551^{abcx} ± 0.30	34.861^{abx} ± 0.45	30.075^{cz} ± 0.40	33.163^{aby} ± 0.38
G – VII (Feed restriction + Ascorbic acid)	99.481^{ax} ± 1.73	86.554^{by} ± 3.83	95.650^{ax} ± 1.43	33.590^{ax} ± 0.86	31.349^{by} ± 0.36	31.807^{bcy} ± 0.49	33.734^{abx} ± 0.34	36.803^{ax} ± 1.97	33.276^{abx} ± 0.27

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

Mean ± SE (between periods) bearing different superscripts (x, y, z) in rows for each parameter differ significantly (P<0.05)

Among the periods there was no significant influence of age on MCHC value of birds in control group (G-I) and in floor space reduced groups (G-II, G-III, G-IV) and in feed restricted group G-VII. The birds of feed restricted groups G-V and G-VI the MCHC value at fourth week of age was significantly (P<0.05) higher than that of sixth and eighth week MCHC value (table 4c).

4.1.7 Differential leucocyte count (DLC)

4.1.7.1 Lymphocytes

At fourth week of age there was no significant variation in lymphocyte counts in birds of floor space reduced group G-II (63.500 ± 0.78) and that of control (G-I) group (63.125 ± 0.44) and also no significant difference was observed in lymphocyte counts in birds of feed restricted group G-V (63.500 ± 0.42) and that of control (G-I) group. Dietary supplementation of Zeetress® and ascorbic acid did not show any significant influence on lymphocyte counts of floor space reduced groups (G-III and G-IV) or feed restricted group (G-VI and G-VIII). At fourth week of age highest lymphocyte count was recorded in feed restricted groups G-VI (64.25 ± 0.53%) and G-VII (64.00 ± 0.46%) whereas floor space reduced group G-IV with dietary supplementation of ascorbic acid had the lowest count (62.075 ± 0.57 %) (table 4d).

At sixth week of age there was significant (P<0.05) variation in lymphocyte counts within groups. The birds of control group G-I had significantly (P<0.05) higher count (63.250 ± 0.49 %) and significantly (P<0.05) lower value was recorded in birds of G-V group (52.000 ± 0.50%).

The Zeetress® and ascorbic acid supplementation in diet significantly ($P<0.05$) increased the lymphocyte counts in birds of floor space reduced groups (G-III) and feed restricted groups (G-VI and G-VII) compared to respective birds of floor space reduced (G-II) and feed restricted (G-V) groups but without any dietary antistress agent (table 4d).

At eighth week of age significant ($P<0.05$) variation in lymphocyte count was recorded among various groups. The control group G-I had significantly ($P<0.05$) highest lymphocyte count ($63.375 \pm 0.50\%$) when compared to feed restricted groups (G-V, G-VI and G-VII) and floor space reduced groups (G-II, G-III and G-IV). The Zeetress® and ascorbic acid supplemented groups showed increased lymphocyte count but was significant ($P\leq0.05$) only in floor space reduction stress groups (G-III and G-IV respectively) as shown in table 4d.

As the lymphocyte count at different age was compared the birds of control group G-I showed an increasing trend with advancement of age. Among the birds of floor space reduced groups G-IV had no significant variation in the lymphocyte count with the advancement age. There was significant ($P<0.05$) influence of age on the lymphocyte count of birds of groups G-II, G-V, G-VI and G-VII and the value showed a decreasing trend with the advancement of age as given in table 4d.

4.1.7.2 Heterophils

At fourth week of age, there was no significant difference in heterophil counts of control group G-I with that birds in floor space reduced groups (G-II, G-III and G-IV) and feed restricted groups (G-V, G-VI and G-VII). Among floor space reduced groups G-II, G-III and G-IV there was no significant variation in heterophil counts. Birds of feed restricted group G-VII with dietary supplementation of ascorbic acid had the lowest (25.750 ± 0.53%) heterophil count and among all the groups the birds of G-IV group (floor space reduction with dietary supplementation of ascorbic acid) had the highest (27.375 ± 0.33%) heterophil count.

At sixth week age there was significant ($P<0.05$) variation in heterophil count of different experimental groups with highest value (37.250 ± 0.31%) in birds of group G-V (feed restricted without any dietary antistress agent and than in G-II group floor space reduced without any dietary antistress agent (33.500 ± 0.57%). The birds of control group (G-I) had a significantly ($P<0.05$) the lowest heterophil count (28.00 ± 0.38%). The dietary supplementation of Zeetress® (G-III and G-VI groups) and ascorbic acid (G-IV and G-VII groups) had significantly ($P<0.05$) lower heterophil count when compared to respective floor space reduced (G-II) and feed restricted (G-V) groups without any dietary antistress agent (table 4d).

At eighth week of age a similar pattern was as observed in various groups as observed at sixth week of age (table 4d).

Among the periods there was significant influence of age on heterophil counts in floor space reduced (G-II, G-III and G-IV) and feed restricted groups (G-V, G-VI and G-VII) as given in table 4d).

4.1.7.3 Monocytes

Monocyte counts (%) for various groups at fourth, sixth, eighth week are given in table 4e. At fourth week of age there was no significant difference in monocyte count within groups with values ranging from 7.125 ± 0.59 % in birds of G-VI group (feed restricted with dietary supplementation of Zeetress®) to 7.500 ± 0.60% in birds of G-VII group (feed restricted supplement with ascorbic acid).

At sixth week of age there was no significant difference ($P>0.05$) in monocyte count in birds of control group G-I with that of floor space reduced groups (G-II, G-III and G-IV) and in birds of groups G-V, G-VI and G-VII (with and without antistress agent). The birds of G-III groups (floor space reduced group with dietary supplementation of Zeetress®) had the lowest (5.250 ± 0.31%) and G-VI group (feed restricted with dietary supplementation of Zeetress® had the highest monocyte count (7.125 ± 0.29 %).

At eighth week of age the birds of G-I group (control) had significantly ($P<0.05$) the lowest value (7.250 ± 0.31%) compared to various experimental groups. The birds of G-IV and G-VII group with dietary supplementation of ascorbic acid) had highest monocyte count (8.375 ± 0.37% and 8.375 ± 0.26% respectively).

Between periods the birds of groups G-III, G-IV and G-VII group floor space reduced but supplemented with antistress agent (feed restricted had significantly ($P<0.05$) lower monocyte count at sixth week of age. There was no significant influence of age on monocyte counts of G-I, G-II, G-V, G-VI groups (Table 4e).

4.1.7.4 Eosinophils

The eosinophil counts for four, six and eight weeks of age are given in Table 4e. There was no significant ($P>0.05$) variation among various groups at fourth, sixth and eighth weeks in eosinophil values. Among the periods too there was no significant variation ($P>0.05$) on eosinophil count in all the experimenal groups. The eosinophil count ranged between 1.625 ± 0.26 % to 2.125 ± 0.29 % (table 4e).

4.1.7.5 Basophils

The basophil count at fourth week, sixth week and eighth week of age for different groups are given in Table 4e. At fourth week of age there was no significant variation ($P<0.05$) in basophil count among the groups with basophil count ranging from $0.750 \pm 0.16\%$ in birds of control group G-I to $0.875 \pm 0.35\%$ in birds of floor space reduced group G-II.

At sixth week of age the basophil count was significantly ($P<0.05$) highest (2.500 ± 0.19 %) in birds of G-II groups floor space reduced and in G-V group (2.125 ± 0.23 %) feed restricted group G-V without any dietary antistress agent. The dietary supplementation of Zeetress® had significantly ($P<0.05$)

lower the basophil count in the birds of both floor space reduced group (G-III) and feed restricted group (1.125 ± 0.23% G-VI 1.375 ± 0.18% respectively) whereas ascorbic acid supplementation decreased basophil count not only in floor space reduced group G-IV (1.125 ± 0.23%) but with significant decrease only in birds of feed restricted group G-IV (1.500 ± 0.19%).

At eighth week of age the basophil count was significantly ($P<0.05$) highest in birds of feed restricted group G-V (2.375 ± 0.29 %) and floor space reduced group G-II (2.00 ± 0.33%) without any dietary antistress agent when compared to birds of control group G-I (1.125 ± 0.12 %). The Zeetress and ascorbic acid supplementation significantly ($P<0.05$) reduced the basophil count in birds of both floor space reduced groups (G-III and G-VI) and feed restricted groups (G-VI and G-VII) (table 4e).

Among the periods, there was no significant influence of age on basophil count in birds of control group G-I, G-III group and G-IV group (floor space reduction with supplementation of antistress agent) and G-VII group (feed restriction with supplementation of ascorbic acid). However, in birds of floor space reduced group G-II, feed restricted group G-V and in G-VI group supplement with Zeetress® the fourth week of age had significantly ($P<0.05$) lower values as compared to sixth and eighth week values (Table 4d).

4.1.8 Heterophil: Lymphocyte ratio

At fourth week of age there was no significant variation in heterophil: lymphocyte (H:L) ratio in birds of floor space reduced group G-II or feed restricted group (G-V) when compared to that of control birds (G-I). The highest H:L ratio of 0.439 ± 0.01 was recorded in floor space reduced group G-IV with dietary supplementation of ascorbic acid and the H:L ratio was lowest (0.403 ± 0.01) for feed restricted group G-VII with dietary supplementation of ascorbic acid (table 4d and fig.1).

At sixth weeks of age significantly ($P<0.05$) higher H:L ratio was observed in birds of feed restricted group G-V without any dietary antistress age (0.717 ± 0.01) and in floor space reduced group G-II without any dietary antistress agent (0.605 ± 0.01) when compared to control group G-I (0.443 ± 0.01). The Zeetress in diet significantly ($P<0.05$) lowered H:L ratio in birds of both floor space reduced group G-III (0.484 ± 0.01) and feed restricted group G-VI (0.510 ± 0.01) similarly the ascorbic acid supplementation also significantly ($P<0.05$) lowered H:L ratio in both in birds of floor space reduced group G-IV (0.446 ± 0.01) and feed restricted group G-VII (0.522 ± 0.01). In general birds of feed restricted groups (G-V, G-VI, G-VII) had relatively higher H:L ratio when compared to birds of floor space reduced groups (G-II, G-III and G-IV) vide Table 4d.

At eight weeks of age H:L ratio showed similar pattern as that of sixth week with significantly ($P<0.05$) higher value for feed restricted group G-V (0.609 ± 0.02) and floor space reduced group G-II without any dietary antistress agent (0.527 ± 0.02). A significantly ($P<0.05$)lower H:L ratio was recorded in birds of control group G-I (0.417 ± 0.01). The Zeetress supplementation in diet resulted in significantly ($P<0.05$) lower H:L ratios for both floor space

Table 4d. Effect of induced stress and antistress agents on Differential leucocyte count (DLC) and Heterophil:Lymphocyte (H:L) ratio of broiler chicken from fourth to eighth week of age Mean ± SE (n=8)

PARAMETER	LYMPHOCYTE (L) [%]			HETEROPHIL [%] (H)			H:L RATIO		
Periods / Groups	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week
G – I (Control)	63.125^{abx} ± 0.44	63.250^{ax} ± 0.49	63.375^{ax} ± 0.50	27.000^{abx} ± 0.27	28.000^{ex} ± 0.38	26.375^{cx} ± 0.80	0.428^{abx} ± 0.00	0.443^{ix} ± 0.01	0.417^{cx} ± 0.01
G – II (Floor space reduction)	63.500^{abx} ± 0.78	55.375^{dz} ± 0.46	57.875^{cy} ± 1.04	26.750^{abcz} ± 0.25	33.500^{bx} ± 0.57	30.375^{by} ± 0.46	0.422^{abz} ± 0.01	0.605^{bx} ± 0.01	0.527^{by} ± 0.02
G – III (Floor space reduction + Zeetress)	63.125^{abx} ± 0.40	62.000^{abxy} ± 0.46	61.125^{by} ± 0.83	26.625^{abcy} ± 0.57	30.000^{dx} ± 0.60	27.875^{cy} ± 0.23	0.422^{aby} ± 0.01	0.484^{cx} ± 0.01	0.457^{cx} ± 0.01
G – IV (Floor space reduction + Ascorbic acid)	62.075^{bx} ± 0.57	60.625^{bx} ± 0.63	61.000^{bx} ± 1.00	27.375^{ay} ± 0.33	30.875^{cdx} ± 0.48	27.875^{cy} ± 0.52	0.439^{ay} ± 0.01	0.510^{dcx} ± 0.01	0.459^{cy} ± 0.01
G – V (Feed restriction)	63.500^{abx} ± 0.42	52.000^{cz} ± 0.50	54.500^{cy} ± 0.73	26.875^{abcz} ± 0.26	37.250^{ax} ± 0.31	33.125^{ay} ± 0.77	0.415^{abz} ± 0.00	0.717^{ax} ± 0.01	0.609^{ay} ± 0.02
G – VI (Feed restriction + Zeetress)	64.25^{ax} ± 0.53	59.000^{cy} ± 0.38	57.250^{cz} ± 0.72	26.125^{bcy} ± 0.61	30.750^{cdx} ± 0.70	32.000^{abx} ± 0.46	0.407^{bz} ± 0.01	0.522^{cdy} ± 0.01	0.560^{bx} ± 0.01
G – VII (Feed restriction + Ascorbic acid)	64.00^{ax} ± 0.46	58.625^{cy} ± 0.50	57.500^{cy} ± 0.53	25.750^{cy} ± 0.53	32.000^{cx} ± 0.33	30.875^{bx} ± 0.61	0.403^{by} ± 0.01	0.546^{cx} ± 0.01	0.538^{bx} ± 0.01

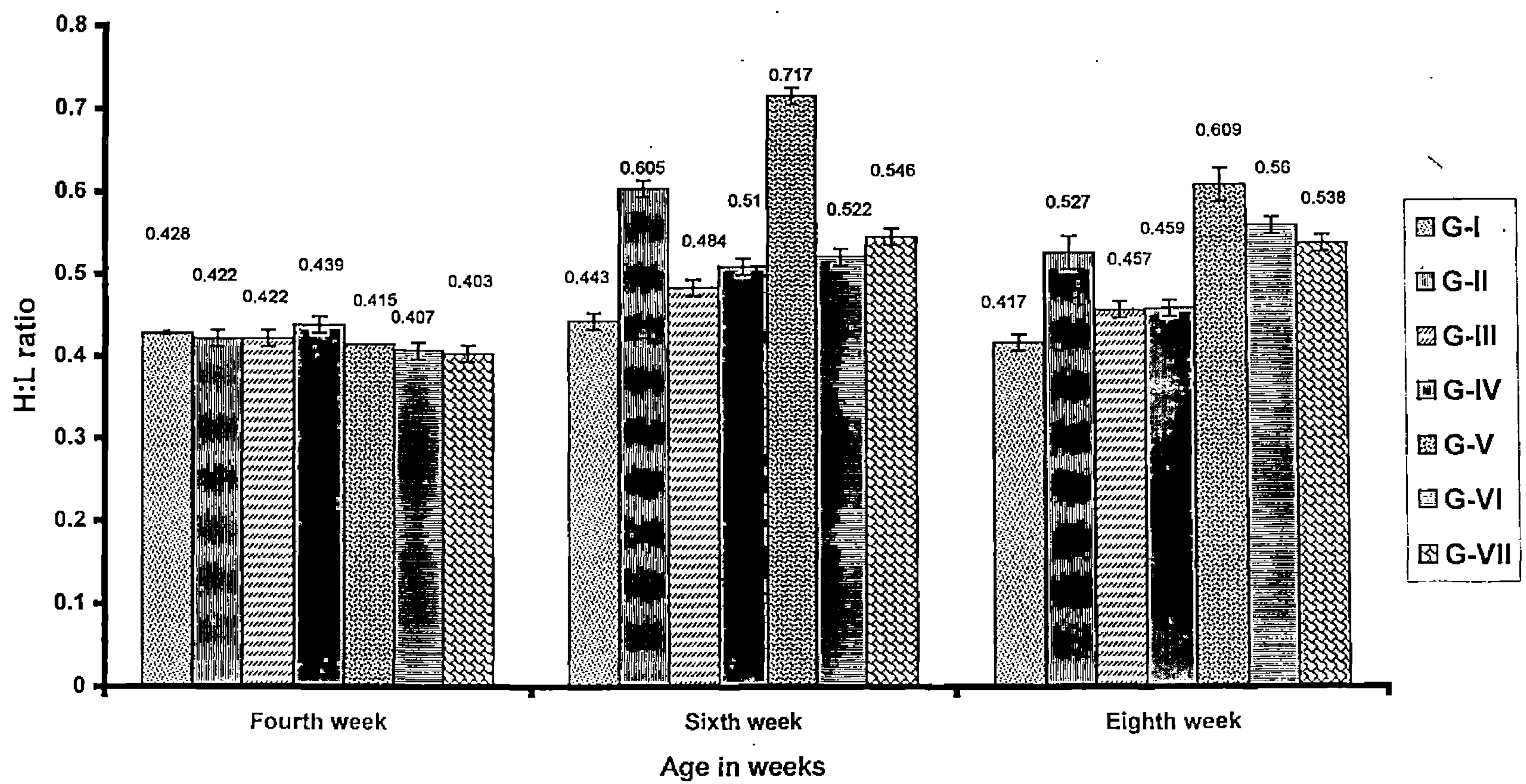
Mean ± SE (between groups) bearing different superscripts (a, b, c, d, e, f) in columns differ significantly ($P<0.05$)
Mean ± SE (between week) bearing different superscripts (x, y, z) in rows for each parameter differ significantly ($P<0.05$)

Table 4e. Effect of induced stress and antistress agents on Differential leucocyte count of broiler chicken from fourth to eighth week of age, Mean ± SE (n=8)

PARAMETER	MONOCYTE [%]			EOSINOPHIL [%]			BASOPHIL [%]		
Periods / Groups	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week
G – I (Control)	7.375^{ax} ± 0.53	6.125^{abcx} ± 0.44	7.250^{bx} ± 0.31	1.750^{ax} ± 0.25	1.750^{ax} ± 0.25	1.625^{ax} ± 0.26	0.750^{ax} ± 0.16	0.875^{cx} ± 0.29	1.125^{cx} ± 0.12
G – II (Floor space reduction)	7.250^{ax} ± 0.65	6.750^{abx} ± 0.45	8.000^{abx} ± 0.27	1.625^{ax} ± 0.26	1.875^{ax} ± 0.29	1.750^{ax} ± 0.31	0.875^{ay} ± 0.35	2.500^{ax} ± 0.19	2.000^{abx} ± 0.33
G – III (Floor space reduction + Zeetress)	7.375^{ax} ± 0.60	5.250^{bcy} ± 0.31	8.125^{abx} ± 0.35	2.000^{ax} ± 0.27	1.625^{ax} ± 0.26	1.750^{ax} ± 0.31	0.875^{ax} ± 0.23	1.125^{cx} ± 0.23	1.125^{cx} ± 0.22
G – IV (Floor space reduction + Ascorbic acid)	7.375^{ax} ± 0.46	5.625^{bcy} ± 0.53	8.375^{ax} ± 0.37	2.000^{ax} ± 0.19	1.750^{ax} ± 0.25	1.500^{ax} ± 0.27	0.875^{ax} ± 0.23	1.125^{cx} ± 0.23	1.250^{cx} ± 0.25
G – V (Feed restriction)	7.250^{ax} ± 0.56	6.750^{abx} ± 0.49	7.875^{abx} ± 0.40	2.000^{ax} ± 0.27	1.750^{ax} ± 0.31	2.125^{ax} ± 0.29	0.875^{ay} ± 0.23	2.125^{abx} ± 0.23	2.375^{ax} ± 0.29
G – VI (Feed restriction + Zeetress)	7.125^{ax} ± 0.59	7.125^{ax} ± 0.29	7.625^{abx} ± 0.37	1.875^{ax} ± 0.22	1.625^{ax} ± 0.18	1.625^{ax} ± 0.29	0.750^{ay} ± 0.25	1.375^{cx} ± 0.18	1.500^{bcx} ± 0.19
G – VII (Feed restriction + Ascorbic acid)	7.500^{ax} ± 0.60	6.125^{abcy} ± 0.29	8.375^{ax} ± 0.26	1.875^{ax} ± 0.23	1.750^{ax} ± 0.16	1.625^{ax} ± 0.33	0.875^{ax} ± 0.31	1.500^{bcx} ± 0.19	1.500^{bcx} ± 0.19

Mean ± SE (between groups) bearing different superscripts (a, b, c, d) in columns differ significantly ($P<0.05$)
Mean ± SE (between periods) bearing different superscripts (x, y) in rows for each parameter differ significantly ($P<0.05$)

Fig. 1 Effect of induced stress and antistress agents on heterophil: lymphocyte (H:L) ratio of broiler chicken from fourth to eighth week of age, Mean±SE(n=8)



reduction group G-III (0.457 ± 0.01) and feed restricted group G-VI (0.560 ± 0.01). Similarly ascorbic acid supplementation also significantly ($P<0.05$) lowered H:L ratio in floor space reduced group G-IV (0.459 ± 0.01) and G-VII (0.538 ± 0.01) when compared with their corresponding floor space reduced group G-II and feed restricted group G-V without any antistress agents (table 4d and fig.1).

Among the periods there was significant ($P<0.05$) influence of age on H:L ratio in almost all the groups with the exception of control group G-I. In birds of control group G-I, floor space reduced groups (G-II, G-III and G-IV) and feed restricted groups (G-V and G-VII) the peak H:L ratio was recorded at sixth week of age where as in feed restricted group G-VI with dietary supplementation of ascorbic acid had significantly ($P<0.05$) higher H:L ratio at eighth week of age (table 4d and fig.1).

4.1.9 Mitogen induced lymphocyte blastogenic response in broiler chicken

The effect of induced stress and antistress agents on mitogen induced lymphocyte blastogenic response in broiler chicken at fourth, sixth and eighth week of age are given in table 4f and fig.2.

After 72 h of incubation, the isolated lymphocytes responded to plasma of fourth week old birds (initial period of experiment) to the extent of 0.917×10^5 to 0.966 x 105 cells per 500 μl medium from a base value of 0.5 x 105 cells per 500 μl.

At sixth week of age the mitogen induced blastogenic response was considerably inhibited in feed restricted group G-V (0.513 x 10^5 cells per 500 µl) and floor space reduced group G-II (0.660 x 10^5 cell per 500 µl) without any dietary antistress agents, when compared to lymphoblastogenic response of control group G-I (0.963 x 10^5 cells per 500 µl).

At eighth week of age the mitogen induced blastogenic response was considerably inhibited in feed restricted group G-V (0.673 x 10^5 cells per 500 µl) and floor space reduced group G-II (0.730 x 10^5 cells per 500 µl) without any dietary antistress agents, when compared to that of control group G-I (0.947 x 10^5 cells per 500 µl).

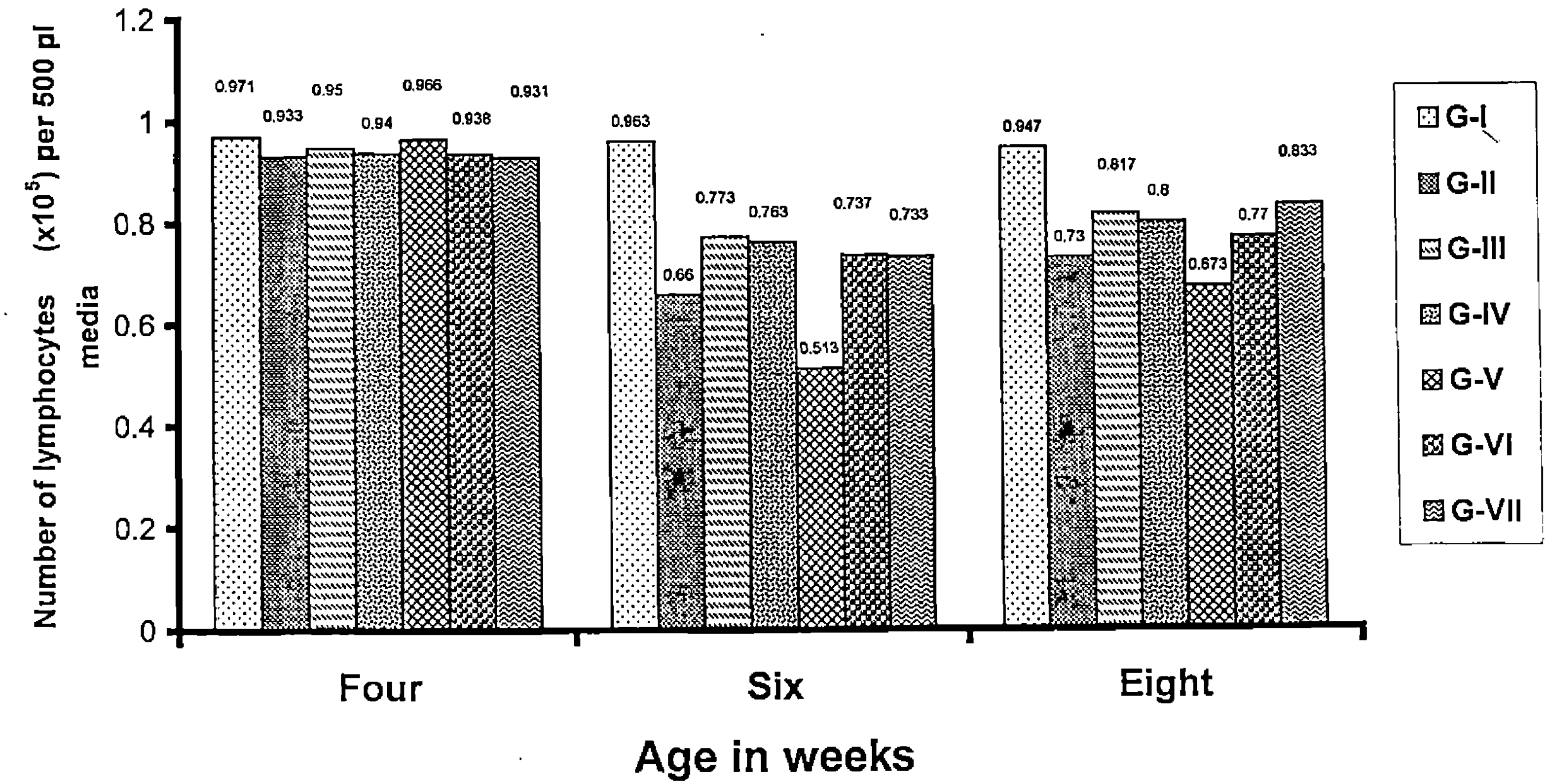
At sixth and eighth week of age dietary supplementation of Zeetress and ascorbic acid considerably increased the mitogen induced lymphocyte blastogenic response in both floor space reduced groups (G-III and G-IV) and feed restricted groups (G-VI and G-VII) when compared to respective floor space reduced group G-II and feed restricted group G-V without any dietary antistress agents.

Among the periods, in control group G-I there was no considerable influence of age on lymphoblastogenic response at fourth, sixth and eighth week of age with highest value at sixth week of age. In all other groups from G-II to G-VII value was lowest at sixth week of age and highest at fourth week of age (table 4f and fig.2).

Table 4f. Effect of induced stress and antistress agent on mitogen induced lymphocyte blastogenic response in broiler chicken after 72 h of incubation

Parameters	Number of lymphocytes (x10^5) per 500 μl media		
Groups \ Periods	Fourth week	Sixth week	Eighth week
G – I (Control)	0.917	0.963	0.947
G – II (Floor space reduction)	0.933	0.660	0.730
G – III (Floor space reduction + Zeetress)	0.950	0.773	0.817
G – IV (Floor space reduction + Ascorbic acid)	0.940	0.763	0.800
G – V (Feed restriction)	0.966	0.513	0.673
G – VI (Feed restriction + Zeetress)	0.938	0.737	0.770
G – VII (Feed restriction + Ascorbic acid)	0.931	0.733	0.833

Fig. 2 Effect of induced stress and antistress agents on mitogen induced lymphocyte blastogenic response in broiler chicken after 72 h of incubation.



4.2. Effect of induced stress and antistress agents on biochemical parameters

4.2.1 Plasma protein profile

4.2.1.1 Total plasma proteins

The total plasma protein concentration for the seven groups at fourth, sixth and eighth week of age are given in table 5a.

At fourth week of age there was no significant variation (P>0.05) in the total plasma protein concentration within groups. The birds of control group G-I had lowest total plasma protein concentration (5.288 ± 0.11 g/dl) and feed restricted group G-VII with dietary ascorbic acid supplementation had the highest total plasma protein value (5.432 ± 0.10 g/dl).

At sixth week of age there was no significant difference in total plasma protein value of birds of various experimental groups. The birds in floor space reduced group G-II had the highest total plasma protein concentration (5.760 ± 0.13 g/dl) and birds of feed restricted group G-VII with dietary supplementation of ascorbic acid had the lowest total plasma protein concentration (5.290 ± 0.18 g/dl).

At eight week age there was no significant difference (P>0.05) in plasma protein concentration of control group G-I with that of floor space reduced group G-II or feed restricted group G-V without any dietary antistress agent. The feed restricted group G-V without any dietary antistress agent had the lowest total plasma protein concentration (5.132 ± 0.16 g/dl) and in floor had albumin concentration of 2.565 ± 0.15 g/dl. The birds of floor space reduced group G-II without any dietary antistress agent (2.668 ± 0.05 g/dl) had the highest albumin concentration whereas the birds of feed restricted group G-V without any antistress agent had the lowest albumin concentration (2.380 ± 0.06 g/dl).

At eighth week of age also, there was no significant difference in plasma albumin concentration in birds control group G-I with that of floor space reduced group G-II or feed restricted group G-V without any antistress agent. Dietary Zeetress® supplementation significantly ($P<0.05$) lowered the plasma albumin concentration in birds of floor space reduced group G-III whereas the decrease in feed restricted group G-VI was non-significant and dietary ascorbic acid supplementation had no significant influence on albumin value both in floor space reduced (G-IV) or in feed restricted (G-VII) groups when compared to values of respective groups G-II and G-V without any dietary antistress agent. The highest albumin concentration (2.775 ± 0.07 g/dl) was observed in birds of G-II group (floor space reduced without any dietary antistress agent) and a lowest (2.225 ± 0.06 g/dl) albumin value was observed in birds of G-VI group (feed restricted with dietary supplementation of Zeetress®).

There was no significant influence of age on albumin concentration at fourth, sixth and eighth week of age in control group G-I, floor space reduced groups G-III, G-IV and feed restricted groups G-V, G-VI and G-VII. In birds of floor space reduced group G-II a significantly ($P<0.05$) lower fourth week albumin concentration was recorded as given in table 5a.

4.2.1.3 Plasma globulin

The fourth, sixth and eighth week value of plasma globulin concentration for seven treatment groups are given in table 5a.

At fourth week of age there was no significant variation in plasma globulin concentration among the various groups. The highest plasma globulin value was recorded in birds of control group G-I (3.030 ± 0.11 g/dl) and lowest globulin concentration was observed in birds of feed restricted group G-V without any dietary antistress agent (2.829 ± 0.10 g/dl).

At sixth week of age there was no significant difference in globulin concentration among the various groups. The birds of control group G-I had a value of 2.992 ± 0.10 g/dl. The floor space reduced groups G-II, G-III and G-IV had comparatively higher globulin values (3.092 ± 0.09 g/dl, 3.092 ± 0.12 g/dl and 3.014 ± 0.09 g/dl respectively) when compared with feed restricted groups G-V, G-VI and G-VII (2.937 ± 0.12 g/dl, 2.935 ± 0.12 g/dl and 2.851 ± 0.9 g/dl respectively).

At eighth week of age too there was no significant variation in plasma globulin values of control group G-I with that of floor space reduced groups (G-II, G-III and G-IV) or feed restricted groups (G-V, G-VI and G-VII). The birds of floor space reduced group G-IV with dietary ascorbic acid supplementation had the highest (3.353 ± 0.06 g/dl) and feed restricted group without any antistress agents G-V had the lowest (2.704 ± 0.13 g/dl) globulin value.

Among the periods, there was no significant influence of age on globulin values of control group G-I, floor space reduced groups G-II and G-III feed restricted groups G-V, G-VI and G-VII. The birds of floor space reduced group G-IV without any dietary antistress agent the eighth week plasma globulin value (3.353 ± 0.06 g/dl) was significantly higher when compared to their fourth (2.958 ± 0.16 g/dl) and sixth (3.014 ± 0.09 g/dl) week values as given in table 5a.

4.2.1.4 Albumin: Globulin ratio

The Albumin: globulin (A:G) ratio at fourth, sixth and eighth week of age for the seven treatment groups are given in table 5a.

At fourth, sixth and eighth week of age there was no significant effect of induced stress and antistress agent on A:G ratio of broiler chicken. At fourth week of age the lowest A:G ratio of 0.757 ± 0.05 was observed in birds of control group G-I and the highest ratio of 0.887 ± 0.06 was recorded in birds of feed restricted group G-V without any dietary antistress agent.

At sixth week of age lowest A:G ratio of 0.818 ± 0.04 was recorded in birds of feed restricted group G-V without any dietary antistress agent and highest ratio of 0.870 ± 0.02 was observed in birds of floor space reduced group (G-IV) with dietary supplementation of ascorbic acid.

At eighth week age higher A:G ratio was recorded in birds of floor space reduced group G-II (0.958 ± 0.05) and (feed restricted group G-V without any dietary antistress agent) (0.910 ± 0.05), whereas in birds of G-IV group floor

Table 5a. Effect of induced stress and antistress agents on plasma protein profile of broiler chicken from fourth to eighth week of age, Mean ± SE (n=8)

PARAMETER	Total plasma proteins (g/dl)			Plasma albumin (g/dl)			Plasma globulin (g/dl)			Albumin:Globulin ratio		
Periods / Groups	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week
G – I (Control)	5.288^{ax} ± 0.11	5.556^{ax} ± 0.24	5.540^{abx} ± 0.16	2.258^{ax} ±0.09	2.565^{ax} ±0.15	2.594^{abx} ±0.12	3.030^{ax} ±0.11	2.992^{ax} ±0.10	2.946^{abx} ±0.12	0.757^{ax} ±0.05	0.853^{ax} ±0.03	0.891^{abx} ±0.05
G – II (Floor space reduction)	5.304^{ax} ± 0.12	5.760^{ax} ± 0.13	5.731^{ax} ± 0.23	2.425^{ay} ±0.11	2.668^{ax} ±0.05	2.775^{ax} ±0.07	2.879^{ax} ±0.07	3.092^{ax} ±0.09	2.956^{abx} ±0.18	0.850^{ax} ±0.05	0.865^{ax} ±0.02	0.958^{ax} ±0.05
G – III (Floor space reduction + Zeetress)	5.312^{ax} ± 0.12	5.733^{ax} ± 0.19	5.415^{abx} ± 0.21	2.428^{ax} ± 0.13	2.641^{ax} ±0.08	2.446^{bcx} ±0.10	2.884^{ax} ±0.10	3.092^{ax} ±0.12	2.970^{abx} ±0.19	0.855^{ax} ±0.07	0.859^{ax} ±0.03	0.851^{abx} ±0.07
G – IV (Floor space reduction + Ascorbic acid)	5.359^{ay} ± 0.19	5.632^{axy} ± 0.16	5.836^{ax} ± 0.08	2.401^{ax} ±0.10	2.618^{ax} ±0.08	2.483^{abcx} ±0.11	2.958^{ax} ±0.16	3.014^{ay} ±0.09	3.353^{ax} ±0.06	0.825^{axy} ±0.05	0.870^{ax} ±0.02	0.745^{by} ±0.04
G – V (Feed restriction)	5.301^{ax} ± 0.10	5.317^{ax} ± 0.14	5.132^{bx} ± 0.16	2.473^{ax} ±0.11	2.380^{ax} ±0.06	2.428^{bcx} ±0.08	2.829^{ax} ±0.10	2.937^{ax} ±0.12	2.704^{bx} ±0.13	0.887^{ax} ±0.06	0.818^{ax} ±0.04	0.910^{ax} ±0.05
G – VI (Feed restriction + Zeetress)	5.340^{ax} ± 0.11	5.398^{ax} ± 0.18	5.320^{abx} ± 0.17	2.340^{ax} ±0.13	2.463^{ax} ±0.08	2.255^{cx} ±0.06	3.000^{ax} ±0.05	2.935^{ax} ±0.12	3.065^{abx} ±0.14	0.784^{ax} ±0.05	0.846^{ax} ±0.04	0.746^{bx} ±0.04
G – VII (Feed restriction + Ascorbic acid)	5.432^{ax} ± 0.10	5.290^{ax} ± 0.18	5.469^{abx} ± 0.23	2.505^{ax} ±0.08	2.439^{ax} ±0.10	2.523^{abcx} ±0.12	2.927^{ax} ±0.08	2.851^{ax} ±0.09	2.947^{abx} ±0.13	0.862^{ax} ±0.04	0.856^{ax} ±0.02	0.859^{abx} ±0.03

Mean ± SE (between groups) bearing different superscripts (a, b, c) in columns differ significantly ($P<0.05$)
Mean ± SE (between periods) bearing different superscripts (x, y) in rows for each parameter differ significantly ($P<0.05$)

space reduced with dietary supplementation of ascorbic acid and in G-VI group ((feed restricted with dietary supplementation of Zeetress®) a lower A:G ratio (0.745 ± 0.04 and 0.746 ± 0.04 respectively) was recorded. The control group G-I had an A:G ratio of 0.891 ± 0.05. Dietary Zeetress® and ascorbic acid supplementation failed to show any significant influence on plasma globulin value in both floor space reduced groups (G-III and G-IV) and feed restricted groups when compared to their respective groups G-II and G-V without any dietary antistress agent (table 5a).

Among the periods, there was no significant influence of age on A:G ratio of control group G-I, floor space reduced groups (G-II and G-III) and feed restricted group (G-V, G-VI and G-VII). In birds of G-IV group floor space reduced with dietary supplementation of ascorbic acid had significantly ($P<0.05$) higher A;G ration at sixth week of age when compared to value at and eighth week of age (Table 5a).

4.2.2 Plasma lipid profile

4.2.2.1 Plasma cholesterol

The plasma cholesterol concentration for various experimental groups in the study at fourth, sixth and eighth week of age is given in table 5b. There was no significant variation in plasma cholesterol concentration among various groups at fourth week of age. The birds of control group G-I had the lowest cholesterol concentration of 136.60 ± 7.68 mg/dl and in birds of feed restricted group G-VI with dietary supplementation of Zeetress® had the highest value of 151.29 ± 7.69 mg/dl (Table 5b.)

At sixth week age there was no significant variation in plasma cholesterol of control group G-I with that of floor space reduced group G-II or feed restricted group G-V without any dietary antistress agent. Dietary Zeetress® and ascorbic acid supplementation in floor space reduced groups (G-III and G-IV respectively) showed a significantly ($P<0.05$) lower plasma cholesterol concentration and this decrease in cholesterol concentration was non significant in feed restricted groups (G-VI and G-VII respectively) when compared to respective groups (G-II and G-V) without any dietary antistress agent. The floor space reduced group G-II without any dietary antistress agent had the highest (204.82 ± 6.77 mg/dl) and the lowest cholesterol value (161.30 ± 5.86 mg/dl) was recorded in birds of G-IV group (floor space reduced) with dietary supplementation of ascorbic acid). The birds of control group G-I had a value of 193.07 ± 11.41 mg/dl.

At eighth week of age a significantly ($P<0.05$) lowest cholesterol concentration was observed in birds of feed restricted group G-V without any dietary antistress agent and in birds of floor space reduced group G-IV with dietary supplementation of ascorbic acid (138.31 ± 6.31 mg/dl and 139.89 ± 10.83 mg/dl respectively) when compared to that of control group G-I. There was no significant variation in cholesterol concentration of floor space reduced groups (G-II, G-III and G-IV) and feed restricted groups (G-V, G-VI and G-VII) (table 5b).

Among the periods, there was no significant influence of age on fourth, sixth and eighth week of age on cholesterol concentration in floor space reduced groups G-III and G-IV and in feed restricted group G-VII. Whereas in birds of feed restricted groups G-V and G-VI a significantly ($P<0.05$) higher value was observed at sixth week of age. In control group G-I cholesterol concentration was significantly ($P<0.05$) lower at the fourth week (Table 5b).

4.2.2.2 Plasma triglyceride

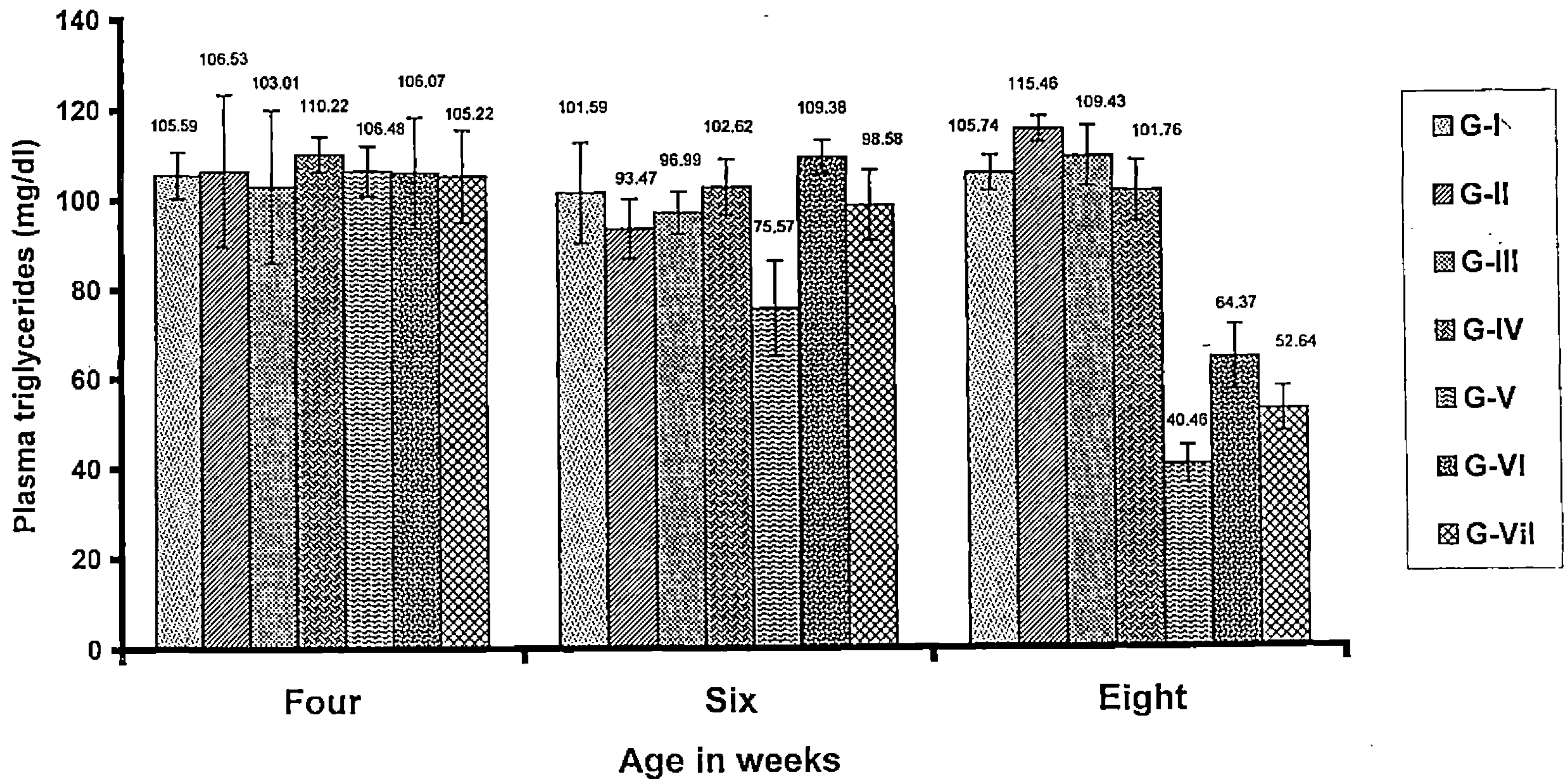
The effect of induced stress and antistress agents on plasma triglycerides concentration in broiler chicken at fourth, sixth and eighth weeks of age are given in table 5b and fig.3. At fourth week there was no significant difference in triglycerides concentration among the birds of various groups. The lowest triglycerides concentration (103.01 ± 17.06 mg/dl) was recorded in birds of floor space reduced group G-III with dietary supplementation of Zeetress® and the highest triglycerides concentration (110.22 ± 3.93 mg/dl) was observed in birds of floor space reduced group G-IV with dietary supplementation of ascorbic acid.

At sixth week of age, there was no significant variation in triglycerides concentration of value of control (G-I) and in all the floor space reduced groups (G-II, G-III and G-IV) without any dietary antistress agent whereas among feed restricted groups the G-V group without any dietary antistress agent had significantly ($P<0.05$) lower (75.57 ± 10.62 mg/dl) triglycerides concentration when compared to that of control group G-I (101.59 ± 11.24 mg/dl).

At eighth week age significantly (P<0.05) the lowest triglycerides value (40.46 ± 4.18 mg/dl) was observed in birds of feed restricted group G-V without any dietary antistress agent when compared to that of control group G-I (105.74 ± 3.97 mg/dl). There was no significant variation in triglycerides concentration of control (G-I) and all floor space reduced groups (G-II, G-III and G-IV). The birds feed restricted groups G-VI (64.37 ± 7.31 mg/dl) and G-VII (52.64 ± 5.04 mg/dl) had significantly (P<0.05) lower triglycerides value when compared to that of control G-I (105.74 ± 3.97 mg/dl) and all the groups G-II (115.46 ± 2.87 mg/dl), G-III (109.43 ± 6.73 mg/dl) and G-IV (101.76 ± 6.78 mg/dl). In both sixth and eighth week of age, dietary Zeetress® and ascorbic acid supplementation failed to show any significant influence on plasma triglycerides value in floor space reduced groups G-III and G-IV, whereas Zeetress® significantly elevated the triglycerides concentration in feed restricted group G-VI and ascorbic acid did not show any significant influence on triglycerides value when compared to values of respective groups G-II and G-V without any dietary antistress agent (table 5b).

Among the periods, there was no significant influence of age on triglycerides values of control (G-I) and floor space reduced groups (G-II, G-III, G-IV). Whereas the birds of feed restricted group G-V without any dietary antistress agent showed a significantly (P<0.05) lower value with the age. However, the birds of feed restricted groups (G-VI and G-VII) showed a significantly (P<0.05) lower triglycerides value at eighth week of age (Table 5b and fig.3).

Fig. 3 Effect of induced stress and antistress agents on plasma concentration of triglycerides in broiler chicken from fourth to eighth week of age, Mean ±SE(n=8)



4.2.2.3 Plasma total lipids

Effect of induced stress and antistress agent on plasma total lipids in the seven treatment groups at fourth, sixth and eight week are given in Table 5b. At fourth week of age there was no significant difference in plasma total lipids value among the various groups. Total lipids concentration was lowest (599.56 ± 10.44 mg/dl) in birds of G-II group (floor space reduced without any dietary antistress agent) and the highest value (618.30 ± 24.85 mg/dl) was recorded birds of G-VIII group (feed restricted with dietary supplementation of ascorbic acid).

At sixth week age the birds of control group G-I had significantly ($P<0.05$) higher (729.41 ± 10.42 mg/dl) plasma total lipids concentration when compared to the feed restricted groups G-V (560.60 ± 2.52 mg/dl) and G-VI (556.23 ± 4.21mg/dl) and G-VII (574.55 ± 12.09 mg/dl) and floor space reduced groups G-II (689.82 ± 21.56), G-III (687.61 ± 15.68) and G-IV (599.55 ± 4.56). Dietary Zeetress® supplementation failed to show any significant influence on total lipid concentration in both G-III and G-VI. Dietary ascorbic acid supplementation in floor space reduced group G-IV resulted in significant ($P<0.05$) decrease in plasma total lipids concentration whereas it had no significant effect in feed restricted group G-VII when compared to their respective groups G-II and G-V without any dietary antistress agent (table 5b).

At eighth week of age the feed restricted group G-V had significantly ($P<0.05$) the lowest (493.77 ± 7.55 mg/dl) total lipid concentration when

Table 5b. Effect of induced stress and antistress agents on plasma lipid profile of broiler chicken from fourth to eighth week of age, Mean ± SE (n=8)

PARAMETER	PLASMA CHOLESTEROL (mg/dl)			PLASMA TRIGLYCERIDES (mg/dl)			PLASMA TOTAL LIPIDS (mg/dl)		
Periods / Groups	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week
G – I (Control)	136.60^{ay} ± 7.68	193.07^{abx} ± 11.41	169.95^{ax} ± 5.21	105.59^{ax} ± 5.21	101.59^{ax} ± 11.24	105.74^{ax} ± 3.97	613.71^{ay} ± 25.12	729.41^{ax} ± 10.42	532.21^{abz} ± 31.03
G – II (Floor space reduction)	147.80^{az} ± 5.39	204.82^{ax} ± 6.77	165.39^{aby} ± 5.14	106.53^{ax} ± 16.90	93.47^{abx} ± 6.70	115.46^{ax} ± 2.87	599.56^{ay} ± 10.44	689.82^{bx} ± 21.56	562.50^{ay} ± 9.98
G – III (Floor space reduction + Zeetress)	144.71^{ax} ± 8.50	166.30^{bcx} ± 10.09	148.49^{abx} ± 9.98	103.01^{ax} ± 17.06	96.99^{abx} ± 4.73	109.43^{ax} ± 6.73	612.35^{ay} ± 25.89	687.61^{bx} ± 15.68	561.42^{ay} ± 17.84
G – IV (Floor space reduction + Ascorbic acid)	144.63^{ax} ± 7.26	161.30^{cx} ± 5.86	139.89^{bx} ± 10.83	110.22^{ax} ± 3.93	102.62^{ax} ± 6.29	101.76^{ax} ± 6.78	616.53^{ax} ± 24.79	599.55^{cx} ± 4.56	580.99^{ax} ± 13.38
G – V (Feed restriction)	139.60^{ay} ± 8.30	180.50^{abcx} ± 6.58	138.31^{by} ± 6.31	106.48^{ax} ± 5.50	75.57^{by} ± 10.62	40.46^{cz} ± 4.18	611.71^{ax} ± 9.17	560.60^{dy} ± 2.52	493.77^{bz} ± 7.55
G – VI (Feed restriction + Zeetress)	151.29^{ay} ± 7.69	176.43^{bcx} ± 11.54	146.91^{aby} ± 4.36	106.07^{ax} ± 12.35	109.38^{ax} ± 3.72	64.37^{by} ± 7.31	599.91^{ax} ± 15.04	556.23^{dy} ± 4.21	569.84^{axy} ± 9.02
G – VII (Feed restriction + Ascorbic acid)	145.26^{ax} ± 5.67	168.16^{bcx} ± 4.62	145.36^{abx} ± 11.77	105.22^{ax} ± 10.30	98.58^{abx} ± 7.86	52.64^{bcy} ± 5.04	618.30^{ax} ± 24.85	574.55^{cdxy} ± 12.09	541.47^{ay} ± 4.65

Mean ± SE (between groups) bearing different superscripts (a, b, c, d) in columns differ significantly (P<0.05)
Mean ± SE (between week) bearing different superscripts (x, y, z) in rows for each parameter differ significantly (P<0.05)

compared to that of control group G-I (532.21 ± 31.03 mg/dl). There was no significant variation in total lipid concentration of control group G-I with that of floor space reduced groups (G-II, G-III and G-IV). There was no significant influence of dietary Zeetress® and ascorbic acid supplementation on plasma total lipids in floor space reduced groups (G-III and G-IV). In feed restricted groups the Zeetress® (G-VI) and ascorbic acid (G-VII) supplementation significantly ($P<0.05$) increased the total lipid concentration (569.84 ± 9.02 mg/dl and 541.47 ± 4.65 mg/dl respectively) when compared to feed restricted group G-V without any dietary antistress agent.

Among the periods, the total lipid concentration was significantly ($P<0.05$) higher in birds of control group G-I and floor space reduced groups G-II and G-III at sixth week of age. Whereas in floor space reduced group G-IV, feed restricted groups G-VI and G-VII a comparatively higher value was recorded at fourth week of age. However, in feed restricted group G-V a significantly ($P<0.05$) the lowest value (493.77 ± 7.55 mg/dl) of plasma total lipids was observed at eight week of age (table 5b).

4.2.3 Plasma glucose

The plasma glucose at fourth week of age did not vary significantly among the various groups. The birds of feed restricted group G-VI with dietary supplementation of Zeetress had the highest value of 236.55 ± 6.55 mg/dl and in floor space reduced group G-IV with dietary supplementation of ascorbic acid had the lowest value (229.84 ± 12.63 mg/dl) (Table 5c).

At sixth week of age there was no significant difference in glucose level of control group G-I with that of floor space reduced groups (G-II, G-III and G-IV) or feed restricted groups (G-V, G-VI and G-VII). The birds of control group G-I had glucose concentration of 220.60 ± 15.21 mg/dl. The birds of feed restricted group G-V without any dietary antistress agent had the lowest (189.99 ± 6.44 mg/dl) glucose concentration. The birds of floor space reduced group G-III with dietary supplementation of Zeetress® had the highest glucose concentration (249.61 ± 9.96 mg/dl). Dietary supplementation of Zeetress® and ascorbic acid in both floor space reduced groups (G-III and G-IV) and feed restricted groups (G-VI and G-VII) failed to show any significant influence on the plasma glucose concentration when compared to the respective groups (G-II and G-V) without any dietary antistress agent (table 5c).

At eighth week of age there was no significant difference (P>0.05) in plasma glucose concentration in various experiment groups with the highest value of 233.60 ± 8.30 mg/dl and lowest value of 204.22 ± 13.79 mg/dl was observed in birds of control group G-I and floor space reduced group G-IV with dietary supplementation of ascorbic acid respectively (table 5c).

Among the periods there was no significant influence (P>0.05) of age in plasma glucose concentration of birds of control group (G-I), floor space reduced groups (G-II, G-III, G-IV) and feed restricted group with dietary ascorbic acid supplementation in diet (G-VII). A significantly (P<0.05) higher value was observed in feed restricted groups (G-V and G-VI) at fourth week of age when compared to the values observed at sixth and eighth week of age (table 5c).

4.2.4 Plasma uric acid

The plasma uric acid concentration of broiler chicken at fourth, sixth and eighth week of age for all seven experiment groups are given in table 5c.

At fourth week of age there was no significant (P>0.05) difference in plasma uric acid concentration among various experiment groups. The lowest plasma uric acid concentration (5.204 ± 0.11 mg/dl) was observed in birds of G-IV group (floor space reduced with ascorbic acid supplementation) and highest plasma uric acid value (6.062 ± 0.31 mg/dl) was recorded in birds of G-VII group (feed restricted with dietary ascorbic acid supplementation).

At sixth week of age birds G-III group (floor space reduced group) with dietary supplementation of Zeetress®) had significantly (P<0.05) higher plasma uric acid concentration (5.584 ± 0.33 mg/dl). The control group (G-I) had a plasma uric acid concentration of 4.914 ± 0.21 mg/dl). The birds of feed restricted groups (G-V, G-VI and G-VII) had significantly (P<0.05) lower plasma uric acid concentration when compared to plasma uric acid concentration of control group G-I. There was no significant difference in plasma uric acid concentration of control group G-I, G-IV group (floor space reduced with dietary supplementation of ascorbic acid and G-II without any dietary antistress agent (table 5c).

At eighth week of age significantly (P<0.05) higher plasma uric acid concentration was observed in birds of feed restricted group G-VI with dietary supplementation of Zeetress® (5.009 ± 0.13 mg/dl) and in control group G-I (4.934 ± 0.13 mg/dl). The floor space reduced group G-II without any antistress agent and feed restricted group G-V without any antistress agent had significantly (P<0.05) lower values (3.323 ± 0.26 mg/dl and 3.511 ± 0.37 mg/dl respectively) when compared to that of control group G-I (4.934 ± 0.13 mg/dl). Zeetress and ascorbic acid supplementation in the diet significantly (P<0.05) improved the plasma uric acid concentration in floor space reduced groups G-III and G-IV (4.136 ± 0.17 mg/dl and 4.710 ± 0.31 mg/dl) and in feed restricted groups G-VI and G-VII (5.009 ± 0.13 mg/dl and 4.501 ± 0.23 mg/dl respectively) when compared to plasma uric acid values of floor space reduction group G-II and feed restricted group G-V without any dietary antistress agents (table 5c).

As the influence of age was compared the values of plasma uric acid at fourth week values were found to be significantly (P<0.05) higher in all the experiment groups. In G-III significantly (P<0.05) lower plasma uric acid concentration was observed at eighth week of age (table 5c).

4.2.5 Plasma cortisol

The effect of induced stress and antistress agents on plasma concentration of cortisol at fourth, sixth and eighth week of age for the seven treatment groups are given in table 5c and fig.4. At fourth week of age within groups there was no significant variation in the level of plasma cortisol. The lowest (3.711 ± 0.39 ng/ml) level of cortisol was observed in birds of feed restricted group G-V without any dietary antistress agent and the highest cortisol concentration (4.121 ± 0.24 ng/ml) was recorded in birds of floor space reduced group G-IV with dietary supplementation of ascorbic acid.

At sixth week of age there was significant (P<0.05) variation in plasma cortisol value within the groups. The feed restricted group G-V and floor space reduced group G-II without any dietary antistress agent had significantly (P<0.05) higher plasma cortisol concentration (6.103 ± 0.22 ng/ml and 5.419 ± 0.32 mg/ml respectively) when compared to that of control group G-I (3.785 ± 0.17 ng/ml) in which cortisol concentration was lowest. In floor space reduced groups G-III and G-IV and in feed restricted groups G-VI and G-VII there was no significant variation in the plasma cortisol values. Dietary supplementation of Zeetress® and ascorbic acid in feed restricted groups (G-VI and G-VII) significantly (P<0.05) lowered plasma cortisol value (5.013 ± 0.17 ng/ml and 5.154 ± 0.32 ng/ml respectively) when compared to feed restricted group G-V without any dietary antistress agent, where as in floor space reduced groups (G-III and G-IV) the effect of antistress agents was non significant.

At eight week of age also the birds of control group G-I had significantly (P<0.05) the lowest (3.914 ± 0.08 ng/ml) plasma cortisol value when compared to all the floor space reduced groups and feed restricted groups. In both floor space reduced (G-III and G-IV) and feed restricted groups (G-VI and G-VII) dietary supplementation of Zeetress and ascorbic acid failed to

Table 5c. Effect of induced stress and antistress agents on plasma biochemical parameters of broiler chicken from fourth to eighth week of age, Mean ± SE (n=8)

PARAMETER	PLASMA GLUCOSE (mg/dl)			CORTISOL (ng/ml)			PLASMA URIC ACID (mg/dl)		
Periods / Groups	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week	Fourth week	Sixth week	Eighth week
G – I (Control)	235.84^{ax} ±11.76	220.60^{abcx} ± 15.21	233.60^{ax} ± 8.30	4.100^{ax} ± 0.04	3.785^{cx} ± 0.17	3.914^{bx} ± 0.08	5.841^{ax} ± 0.38	4.914^{by} ± 0.21	4.934^{ay} ± 0.13
G – II (Floor space reduction)	233.80^{ax} ± 9.14	227.62^{abx} ± 9.11	233.16^{ax} ± 5.96	3.962^{ay} ± 0.33	5.419^{abx} ± 0.32	5.000^{ax} ± 0.11	5.717^{ax} ± 0.11	4.380^{bcy} ± 0.18	3.323^{dz} ± 0.26
G – III (Floor space reduction + Zeetress)	230.10^{ax} ± 14.74	249.61^{ax} ± 9.96	227.73^{ax} ± 13.01	3.885^{ay} ± 0.48	4.756^{bx} ± 0.14	4.725^{ax} ± 0.20	5.762^{ax} ± 0.38	5.584^{ax} ± 0.33	4.136^{bcy} ± 0.31
G – IV (Floor space reduction + Ascorbic acid)	229.84^{ax} ± 12.63	231.81^{abx} ± 12.80	229.62^{ax} ± 13.39	4.121^{ay} ± 0.24	4.875^{bx} ± 0.26	4.665^{ax} ± 0.22	5.204^{ax} ± 0.11	4.375^{bcy} ± 0.09	4.710^{aby} ± 0.17
G – V (Feed restriction)	233.36^{ax} ± 10.11	189.99^{cy} ± 6.44	212.90^{axy} ± 8.00	3.711^{az} ± 0.39	6.103^{ax} ± 0.22	5.250^{ay} ± 0.12	5.900^{ax} ± 0.31	4.211^{cy} ± 0.08	3.511^{cdy} ± 0.37
G – VI (Feed restriction + Zeetress)	236.55^{ax} ± 6.55	215.59^{bcxy} ± 5.83	204.22^{ay} ± 13.79	4.070^{ay} ± 0.06	5.013^{bx} ± 0.17	4.870^{ax} ± 0.10	5.841^{ax} ± 0.38	4.227^{cy} ± 0.26	5.009^{ay} ± 0.13
G – VII (Feed restriction + Ascorbic acid)	232.99^{ax} ± 11.22	200.96^{bcx} ± 7.45	225.67^{ax} ± 12.05	4.069^{ay} ± 0.44	5.154^{bx} ± 0.32	4.688^{ax} ± 0.13	6.062^{ax} ± 0.31	3.788^{cz} ± 0.08	4.501^{aby} ± 0.23

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

Mean ± SE (between periods) bearing different superscripts (x, y, z) in rows for each parameter differ significantly (P<0.05)

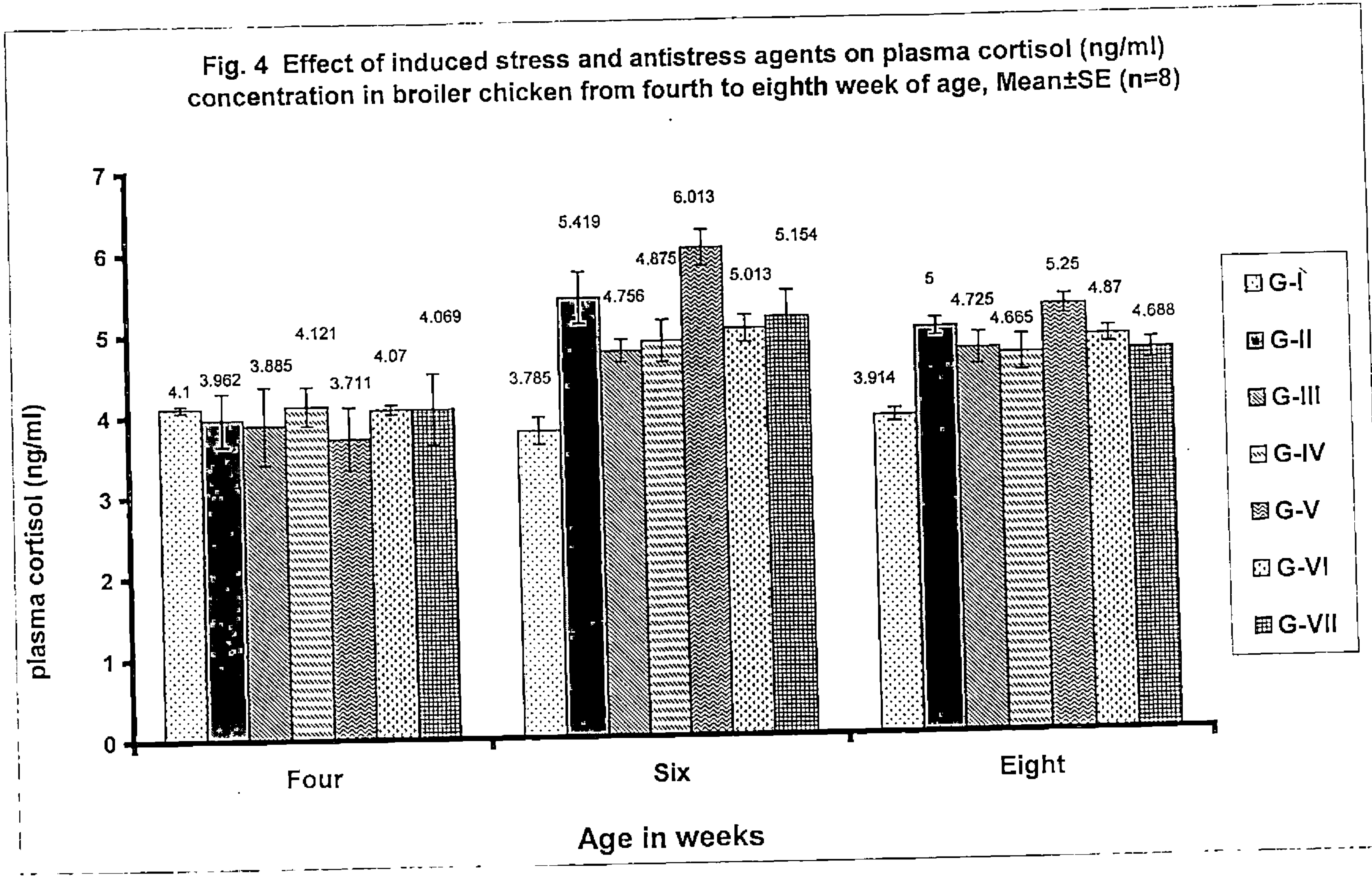


Fig. 4 Effect of induced stress and antistress agents on plasma cortisol (ng/ml) concentration in broiler chicken from fourth to eighth week of age, Mean±SE (n=8)
plasma cortisol (ng/ml)
7
6
5
4
3
2
1
0
4.1
3.962
3.885
4.121
3.711
4.07
4.069
3.785
5.419
4.756
4.875
6.013
5.013
5.154
3.914
5
4.725
4.665
5.25
4.87
4.688
Four
Six
Eight
Age in weeks
G-I
G-II
G-III
G-IV
G-V
G-VI
G-VII

show any significant influence on plasma cortisol value when compared to their respective stressed groups (G-II and G-V) without any dietary antistress agent (table 5c and fig.4).

Among the periods, the plasma cortisol value was significantly ($P<0.05$) the lowest at fourth week of age than that of sixth and eighth week of age in almost all the treatment groups with exception of control group G-I, in which was observed no significant influence of age on plasma cortisol value (table 5c and fig.4).

4.3. Effect of induced stress and antistress agent on production parameters

There was no mortality recorded in any of the groups from fourth to eight week of observation in any of the groups used in the study.

4.3.1. Body weight

The initial body weight (fourth week of age) of birds did not differ significantly among the various groups. At fifth week of age the feed restricted groups (G-V, G-VI and G-VII) had significantly ($P<0.05$) lower body weight (1150 ± 17.67, 1206.25 ± 37.95 and 1175.00 ± 28.35 g respectively) when compared to birds of control group G-I (1350 ± 62.68 g). There was no significant variation in fifth week body weight of control group (G-I) and floor space reduced groups (G-II, G-III and G-IV). The Zeetress® and ascorbic acid supplementation in diets of both floor space reduced (G-III and G-IV) and feed restricted groups (G-VI and G-VII) improved (non-significant) body weight when compared to their respective floor space reduced and feed restricted groups (G-II and G-V) without any dietary antistress agent (table 6a).

At sixth week of age, the birds of feed restricted group (G-V, G-VI and G-VII) had significantly ($P<0.05$) lower body weight when compared with other groups including the control. Zeetress® supplementation in feed restricted group G-VI showed significantly ($P<0.05$) higher body weight whereas the increase in body weight was non significant in birds of ascorbic acid supplemented group G-VII when compared with feed restricted group G-V without any dietary antistress agent. There was no significant difference in body weights of control group G-I, and floor space reduced groups G-II, G-III and G-IV (table 6a).

At seventh week of age, the birds of feed restricted groups, G-V, G-VI and G-VII had significantly ($P<0.05$) lower body weights when compared to control group G-I (2062.50 ± 80.60 g) or floor space reduced groups G-II, G-III and G-IV (Table 6a). There was no significant influence of Zeetress® or ascorbic acid supplementation either in birds of floor space reduced groups; G-III (2037.50 ± 71.18g) and G-IV (2031.25 ± 38.89 g) or in feed restricted groups; G-VI (1725 ± 60.50 g) and G-VII (1625.00 ± 38.96 g) respectively when compared to their respective floor space reduced group G-II (2018.75 ± 61.19 g) and feed restricted group G-V (1581.25 ± 26.42 g) without any dietary antistress agent.

At eighth week of age it showed the same pattern as that of seventh week of age. The highest body weight was recorded in birds of floor space reduced group G-II (2368.75 ± 87.60 g) and lowest for feed restricted group G-V (1787.50 ± 27.59 g).

The effect of induced stress and antistress agents on weekly body weight gain are compared and depicted in table 6a and fig.5 and 6. At fifth week of age a significantly (P<0.05) lower body weight gain was recorded in birds of feed restricted group G-V (215.63 ± 13.21 g), G-VI (246.88 ± 16.79 g) and G-VII (275.00 ± 16.37 g) when compared to control group G-I (390.63 ± 33.93 g) or floor space reduced groups (G-II, G-III and G-IV). The floor space reduced group G-III and G-IV had comparatively higher body weight gain (431.25 ± 23.60 and 421.88 ± 22.29 g). The Zeetress® and ascorbic acid supplementation had no significant influence on body weight gain in both floor space reduced and feed restricted groups although they improved weekly body weight gain.

At sixth week of age the feed restricted group G-V without any dietary antistress agent had significantly (P<0.05) lowest body weight gain (234.75 ± 23.62 g). In feed restricted groups (G-VI and G-VII) dietary Zeetress® and ascorbic acid supplementation showed significant improvement in body weight gain. In all other groups (G-I, G-II, G-III and G-IV) there was no significant variation in body weight gain as given in table 6a and fig.5 and 6.

Table 6a. Effect of induced stress and antistress agents on body weight and weekly body weight gain of broiler chicken from fourth to eighth week of age, Mean ± SE (n=8)

PARAMETER	BODY WEIGHT (g)					WEEKLY BODY WEIGHT GAIN (g)			
Periods / Groups	Fourth week	Fifth week	Sixth week	Seventh week	Eighth week	Fifth week	Sixth week	Seventh week	Eighth week
G – I (Control)	959.25^{a} ± 35.01	1350.00^{a} ± 62.68	1715.63^{a} ± 81.00	2062.50^{a} ± 80.60	2337.50^{a} ± 74.85	390.63^{a} ± 33.93	365.63^{ab} ± 29.86	346.88^{a} ± 25.55	275.00^{ab} ± 28.34
G – II (Floor space reduction)	918.75^{a} ± 30.89	1287.50^{abc} ± 52.40	1693.75^{a} ± 54.64	2018.75^{a} ± 61.19	2368.75^{a} ± 87.60	368.75^{a} ± 28.64	406.25^{a} ± 41.66	325.00^{ab} ± 25.00	350.00^{a} ± 48.18
G – III (Floor space reduction + Zeetress)	884.38^{a} ± 36.89	1315.63^{ab} ± 36.88	1734.38^{a} ± 53.74	2037.50^{a} ± 71.18	2281.25^{a} ± 47.19	431.25^{a} ± 23.60	418.75^{a} ± 33.66	303.13^{abc} ± 26.06	243.75^{b} ± 53.82
G – IV (Floor space reduction + Ascorbic acid)	934.38^{a} ± 39.76	1350.25^{a} ± 42.72	1687.38^{a} ± 31.87	2031.25^{a} ± 38.89	2250.00^{a} ± 42.26	421.88^{a} ± 22.29	331.25^{abc} ± 22.86	343.75^{a} ± 20.13	218.75^{b} ± 33.99
G – V (Feed restriction)	928.13^{a} ± 22.87	1150.00^{c} ± 17.67	1384.38^{c} ± 16.49	1581.25^{b} ± 26.42	1787.50^{b} ± 27.39	215.63^{b} ± 13.21	234.75^{d} ± 23.62	196.88^{d} ± 23.14	206.25^{b} ± 20.61
G – VI (Feed restriction + Zeetress)	959.38^{a} ± 24.98	1206.25^{bc} ± 37.95	1496.25^{b} ± 54.74	1725.00^{b} ± 60.50	1956.25^{b} ± 66.44	246.88^{b} ± 16.79	290.00^{bc} ± 18.79	228.75^{cd} ± 29.06	231.25^{b} ± 9.15
G – VII (Feed restriction + Ascorbic acid)	900.00^{a} ± 31.34	1175.00^{c} ± 28.35	1437.50^{bc} ± 32.39	1625.00^{b} ± 38.96	1837.50^{b} ± 57.28	275.00^{b} ± 16.37	262.50^{c} ± 22.66	187.50^{d} ± 26.30	212.50^{b} ± 24.55

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

Fig. 5 Effect of 33% floor space reduction stress and antistress agents on weekly body weight gain in broiler chicken from fifth to eighth week of age, Mean (n=8)

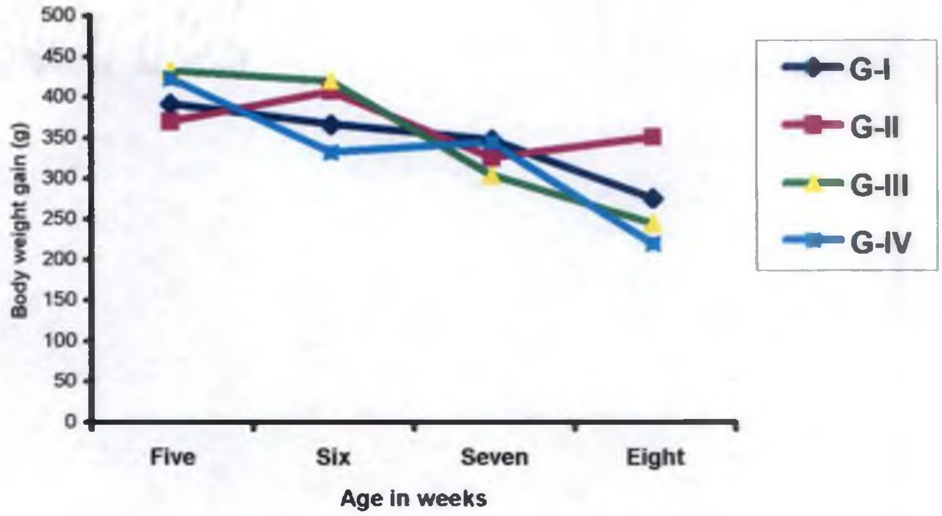
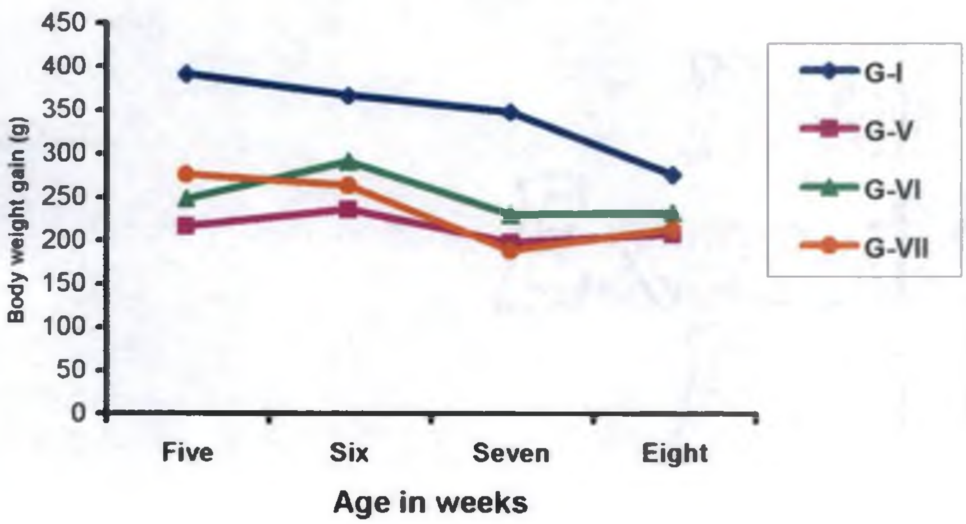


Fig. 6 Effect of 30% feed restriction stress and antistress agents on weekly body weight gain in broiler chicken from fifth to eighth week of age, Mean(n=8)



The seventh week body weight gain did not show any significant variation in birds of control group (G-I) and floor space reduced groups (G-II, G-III and G-IV). The control group G-I had the highest body weight gain (346.88 ± 25.55 g). The feed restricted groups (G-V, G-VI and G-VII) had significantly ($P<0.05$) lower body weight gain when compared to control group (G-I).

At eighth week of age the floor space reduced group G-II recorded the highest body weight gain (350.00 ± 48.18 g) and the feed restricted group G-V without any dietary antistress agent had the lowest body weight gain (206.25 ± 20.61 g). There was no significant variation in body weight gain of birds in control group G-I with that of birds in floor space reduced groups (G-II, G-III and G-IV) or in feed restricted groups (G-V, G-VI and G-VI) as given in table 6a and fig.5 and 6.

4.3.2. Feed consumption/intake

The values of weekly feed intake of various experiment groups (G-I to G-VII) from fifth to eighth week of age are given in table 6b.

4.3.3. Feed efficiency (FE)

Effect of induced stress and antistress agents on feed efficiency (FE) for fifth, sixth, seventh, eighth week and overall (5 to 8 weeks) in birds of various experimental groups are given in table 6b and fig.7. At fifth week the superior FE was observed for floor space reduced group G-III supplemented with

Zeetress® (1.77) and the feed restricted groups G-V had the inferior FE of 2.41. The birds of control group G-I showed a FE of 1.90.

At sixth week of age the best FE was recorded for feed restricted group G-VI with dietary supplementation of Zeetress (1.90) whereas poor FE was observed in birds of floor space reduced group G-IV supplemented with ascorbic acid in diet (2.38).

At seventh week of age the FE value ranged from 2.32 (G-I) to 3.01 (G-VII).

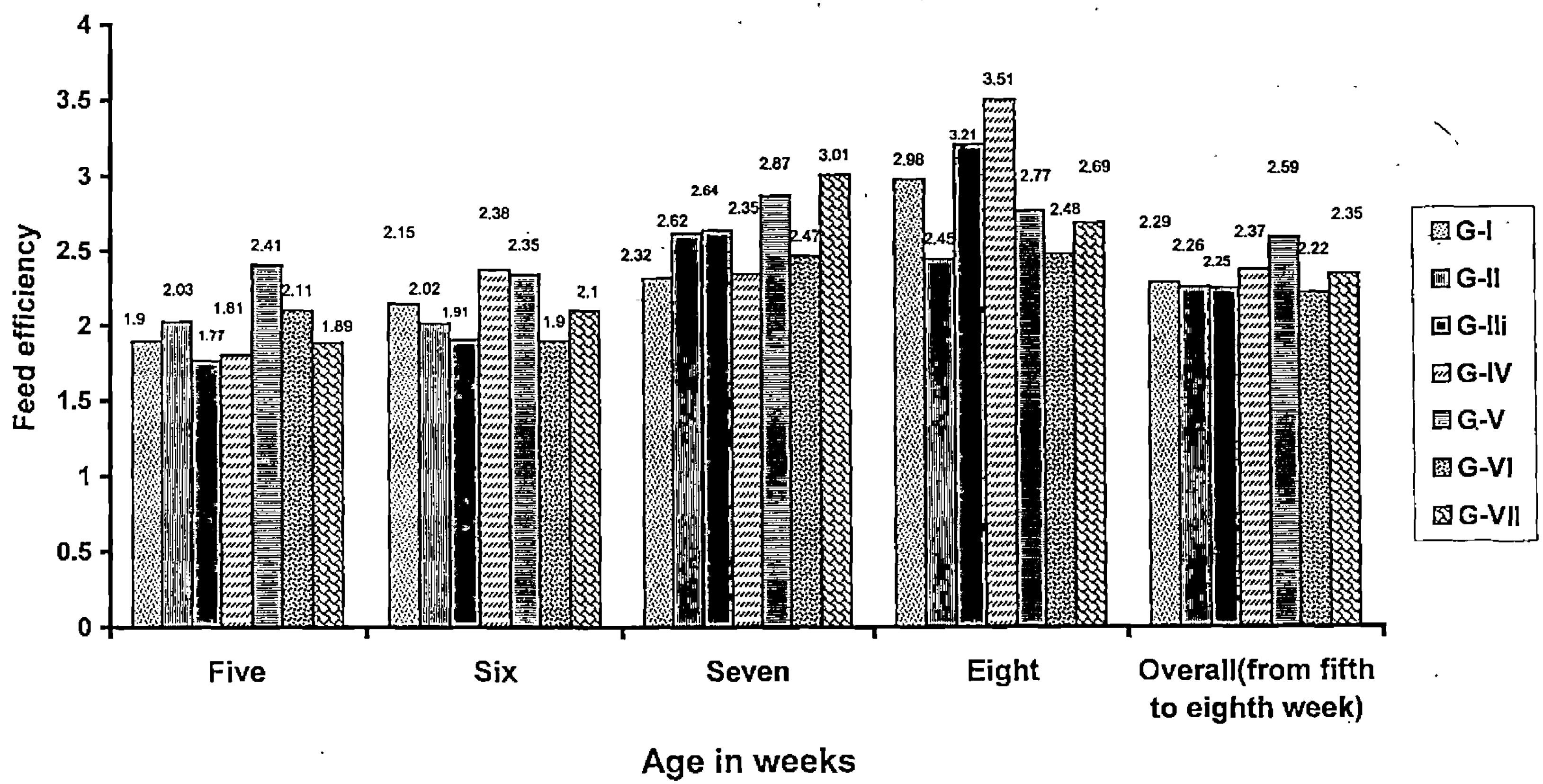
The eighth week FE value ranged from 2.45 in birds of floor space reduced group G-II to 3.51 in floor space reduced group G-IV.

A perusal of the overall FE from 5 to 8 weeks of age revealed that the birds of feed restricted group G-VI with dietary supplementation of Zeetress® recorded a superior FE of 2.22 whereas an inferior value of 2.59 was noted in feed restricted group G-V without any dietary antistress agents. The control group (G-I) recorded a FE of 2.29. Zeetress® supplementation improved overall FE in the feed restricted group, on the other hand this advantage was not observed in the floor space reduced group. Ascorbic acid improved overall FE only in birds of feed restricted group (G-VII) when compared to G-V. However, in birds of floor space reduced group (G-IV) ascorbic acid supplementation could not improve the FE.

Table 6b. Effect of induced stress and antistress agents on feed intake (kg) and feed efficiency of broiler chicken from fourth to eighth week of age

Parameters	Feed intake/week (kg)				Feed efficiency				
Periods / Groups	5^{th} week	6^{th} week	7^{th} week	8^{th} week	5^{th} week	6^{th} week	7^{th} week	8^{th} week	5^{th}-8^{th} week
G – I (Control)	5.950	6.300	6.450	6.550	1.90	2.15	2.32	2.98	2.29
G – II (Floor space reduction)	6.000	6.550	6.800	6.850	2.03	2.02	2.62	2.45	2.26
G – III (Floor space reduction + Zeetress)	6.100	6.400	6.400	6.250	1.77	1.91	2.64	3.21	2.25
G – IV (Floor space reduction + Ascorbic acid)	6.100	6.300	6.450	6.150	1.81	2.38	2.35	3.51	2.37
G – V (Feed restriction)	4.165	4.410	4.515	4.585	2.41	2.35	2.87	2.77	2.59
G – VI (Feed restriction + Zeetress)	4.165	4.410	4.515	4.585	2.11	1.90	2.47	2.48	2.22
G – VII (Feed restriction + Ascorbic acid)	4.165	4.410	4.515	4.585	1.89	2.10	3.01	2.69	2.35

Fig. 7 Effect of induced stress and antistress and antistress agents on feed efficiency of broiler chicken from fifth to eighth week of age.



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Discussion

5. DISCUSSION

5.1 Effect of induced stress and antistress agents on haematological parameters

Evaluation of various haematological parameters depicts an overall picture of health status of an animal/bird and hence any deviation from the normal physiological status will be reflected from the assessment. Haematological dynamics are well selected depending upon the physiological need of birds which vary during stress.

5.1.1 Total erythrocyte count (TEC)

There was no significant variation (P>0.05) in TEC of the birds in floor space reduced group G-II with that of control group (G-I) (table 4a). This may be due to the fact that floor space reduction being a chronic stress (Zulkifli and Siegel, 1995) failed to cause any discernable effect on TEC.

Dietary supplementation of Zeetress® and ascorbic acid failed to show any significant influence (P>0,05) on TEC of the birds in floor space reduced groups G-III and G-IV respectively. However, Sahota *et al.* (1994) reported that ascorbic acid supplementation (50-100 mg/kg feed) significantly improved the TEC in chicken exposed to heat stress.

In the present study the birds of feed restricted group G-V had significantly (P<0.05) lower total erythrocyte count when compared to that of control birds (G-I) both at sixth and eighth week of age (table 4a). This observation was in consonance with findings of Maxwell *et al.* (1991) and they opined that feed restriction of broiler chicken caused a tendency of macrocytic normochromic anaemia, possibly by folic acid deficiency. A severe feed restriction programme may cause even greater anaemia or possibly induce further stress (Maxwell *et al.*, 1990b). Dietary Zeetress® and ascorbic acid supplementation in birds of feed restricted groups G-VI and G-VII respectively failed to improve the TEC, a higher dosage of these antistress agents might probably bring a beneficial effect.

The TEC in the birds of groups viz., G-I, G-II, G-III and G-IV showed a fluctuating trend and the feed restricted groups G-V, G-VI and G-VII showed a decreasing trend with age. There were no such literature available with similar finding for comparison.

5.1.2 Haemoglobin (Hb) and Volume of packed red cells (VPRC)

In present study there was no significant variation in Hb and VPRC values of birds in floor space reduced group G-II from that of control birds of G-I group both at sixth and eighth week of age. This may be due to the level of floor space reduction was not severe enough to cause a marked alteration in Hb and VPRC value. There were no similar reports for comparison.

Dietary Zeetress® and ascorbic acid supplementation failed to show any significant influence on Hb and VPRC values in birds of floor space reduced groups G-III and G-IV respectively. However, Sahota *et al.* (1994) reported that ascorbic acid supplementation significantly improved Hb and VPRC value of chicken exposed to heat stress.

The values of Hb and VPRC in present study were significantly ($P<0.05$) lower in the birds of feed restricted group G-V when compared to that of birds of control group G-I both at sixth and eighth week of age. This observation was in close agreement with findings of Maxwell *et al.* (1990b) who reported that the values of Hb and VPRC were lower in feed restricted birds when compared to *ad libitum* fed birds and according to them some of the erythrocyte characteristics were restored within the "normal" range by feed restriction but with a tendency of microcytic normochromic anaemia in feed restricted birds. These observations are also in support of Donkoh (1989) and Furlan *et al.* (1999) who opined that heat stress was associated with decreased haemoglobin and VPRC values. Dietary Zeetres® and ascorbic acid supplementation in birds of feed restricted groups G-VI and G-VII respectively failed to influence the Hb and VPRC both at sixth and eighth week of age, except a significantly lower value of VPRC in birds of G-VII group at sixth week of age. This may be due to increased water intake in ascorbic acid treated birds. A higher dosage of these antistress agents might have improved the Hb and VPRC in feed restricted birds.

The Hb value of birds of control group G-I, floor space reduced groups G II, G III and G IV and feed restricted group G VI showed a fluctuating trend whereas birds of feed restricted groups G-V and G-VII showed a declining trend with age.

The value of VPRC in birds of control (G-I) and floor space reduced groups (G-II, G-III and G-IV) showed a fluctuating trend and that of feed restricted groups a declining trend was noticed with age. There were no similar reports available in birds for comparison.

5.1.3 Erythrocyte sedimentation rate (ESR)

The ESR value in the present study did not show any significant variation among the birds of various experimental groups except for the birds of feed restricted group G-V at eighth week of age which had significantly ($P<0.05$) lower ESR value when compared to that of birds of control group G-I (table 4b). The ESR values in the present study were in the range of 1.625 ± 0.26 to 2.625 ± 0.18 mm/hr which are in accordance with Sturkie (1965).

Since the avian erythrocytes are biconvex shaped, rouleux formation does not occur and the cells show little tendency to sediment. Hence ESR is not an useful test in case of birds (Arun and Lokhande, 1994). According to Sturkie (1965) the mean sedimentation rates in chicken ranged from 0.5 to 9 mm/hr and opined that most values were between 1.5 and 4 mm/hr.

5.1.4 Total leucocyte count (TLC)

In the present study, there was no significant variation ($P>0.05$) in TLC value in birds of floor space reduced group G-II and that of control group G-I both at sixth and eighth week of age (table 4b). This may be due to the level of floor space reduction was not severe enough to cause a marked alteration in TLC value. Dietary supplement of Zeetress and ascorbic acid failed to show any significant influence on TLC in birds of floor space reduced groups (G-III and G-IV respectively) both at sixth and eighth weeks of age.

The birds of feed restricted group G-V had significantly ($P<0.05$) lower TLC when compared to that of control group G-I (table 4b). This observation is in consonance with earlier findings of Maxwell *et al.* (1990a, 1991) who reported a lower TLC in feed restricted birds. This may be due to a decrease in number of circulating lymphocytes in the birds under stress. In birds stress caused involution of lymphoid tissues such as thymus, spleen and bursa of fabricius (Glick, 1967; Siegel, 1980, 1983). Direct lysis or apoptosis (Munck and Guyre, 1991) and delay in maturation (Sapolsky, 1992) of leucocytes occurred due to induced stress and the increase in corticosteroids further led to the reduction in TLC value. Dietary Zeetress® and ascorbic acid supplementation failed to improve total leucocyte count in the birds of feed restricted groups G-VI and G-VII respectively, a higher dosage of these antistress agents might have proved beneficial. Various kind of stress can affect the TLC value of birds. Lazarevic *et al.* (2000) observed a decrease in TLC in broiler chicken exposed to long term sound stress.

The birds of groups G-I, G-II, G-IV, G-V and G-VI showed a declining trend and G-III and G-VII showed a fluctuating trend with age. No similar reports were available for comparison.

5.1.5 Erythrocyte Indices

5.1.5.1 Mean corpuscular volume (MCV)

In the present study no significant variation (P>0.05) in MCV value of birds of control group G-I and that of floor space reduced group G-II or feed restricted group G-V without any antistress agents both at sixth and eighth week of age. However, Maxwell *et al.* (1990b) reported that MCV value was reduced in feed restricted birds indicating microcytic normochromic anaemia whereas Maxwell *et al.* in 1991 further reported that early age (six day old) feed restriction (for 10-14 days) led to folic acid deficiency which increased the value of MCV in seven week old broiler chicken demonstrating macrocytic normochromic anaemia. In the present study the level of feed restriction or the floor space reduction may not be severe enough to cause a marked variation in the value of MCV in these birds.

Dietary supplementation of Zeetress® had no significant influence on the MCV value either in birds of floor space reduced (G-III) or in feed restricted group (G-VI), both at sixth and eighth week of age. Dietary ascorbic acid supplementation in the birds of feed restricted group G-VII at sixth week of age caused a significantly (P<0.05) lowered MCV value probably due to corresponding lower VPRC at that age when compared to that of G-V without any antistress agent. Whereas in other groups at sixth and eighth week of age it had no significant influence on the MCV value, since there was no corresponding variations in TEC and VPRC in these groups. There were no such earlier reports for comparison.

In birds of the various groups as G-I, G-II, G-III and G-V the MCV values did not vary significantly with age, whereas significantly (P<0.05) lower MCV value was recorded in the birds of groups G-IV and G-VII at sixth week of age and in birds of G-VI group at fourth week of age.

5.1.5.2 Mean corpuscular haemoglobin (MCH)

The MCH value in the present study did not vary significantly in birds of floor space reduced group G-II since there was no corresponding variations in Hb and VPRC values in this group when compared to that of birds of control group G-I both at sixth and eighth week of age. Dietary Zeetress® and ascorbic acid supplementation failed to show any significant influence on MCH values in birds of floor space reduced groups (G-III and G-IV) both at sixth and eighth week of age. There was no significant variation (P>0.05) in MCV value of birds of G-I and feed restricted group G-V at sixth week of age, but a significantly (P<0.05) lower MCH was observed in feed restricted group G-V at eighth week of age. This observation is in close agreement with the findings of Maxwell *et al.* (1990b), who reported a lower MCH value in feed restricted birds. The dietary supplementation of Zeetress® and ascorbic acid failed to show any significant influence on MCH value in feed restricted groups G-VI and G-VII respectively both at sixth and eighth week of age, a higher dosage of these antistress agents might be beneficial. This may also be due to no corresponding variation in Hb value caused by these antistress agents. In birds of groups G-I, G-II, G-III and G-VI there was no significant influence of age on MCH values whereas in G-V and G-VII significantly (P<0.05) higher MCH value was recorded at fourth week of age and in birds of G-VI a significantly (P<0.05) lower MCH value was recorded at sixth week of age. There were no such literature available for comparing the significance of age on erythrocyte indices.

5.1.5.3 Mean corpuscular haemoglobin concentration (MCHC)

In the present study there was no significant variation (P>0.05) in MCHC values in birds of floor space reduced group G-II and control group G-I was observed both at sixth and eighth week of age, since there was no corresponding variations in TEC and Hb values in these groups. Dietary Zeetress® and ascorbic acid supplementation failed to show any significant influence on MCHC value in birds of floor space reduced groups (G-III and G-IV) at sixth and eighth week of age. In the birds of feed restricted group G-V the MCV value did not vary significantly from that of control group G-I. This observation is in consonance with findings of Maxwell *et al.* (1990b) who reported that there was no significant difference in MCHC value in feed restricted birds when compared with *ad libitum* fed birds.

Dietary Zeetress® and ascorbic acid supplementation in birds of feed restricted groups G-VI and G-VII had no significant influence on the MCHC value at eight week of age whereas at sixth week of age the MCHC value was significantly (P<0.05) lower in Zeetress® supplemented birds and higher MCHC value was observed in ascorbic acid supplemented birds when compared to MCHC of birds of G-V group. This observation was due to corresponding variations in TEC and Hb at sixth week of age. The MCHC value in the birds of groups G-I, G-II, G-III, G-IV and G-VII did not vary significantly with age whereas in birds of G-V and G-VI a significantly higher MCHC was observed at fourth week of age (table 4c).

5.1.6 Differential leucocyte count (DLC) and Heterophil: lymphocyte (H:L) ratio

In the present study a significantly (P<0.05) lower lymphocyte count and a significantly (P<0.05) higher heterophil count were observed in birds of floor space reduced group G-II and feed restricted G-V and without any dietary antistress agent both at sixth and eighth week of age with resultant significantly (P<0.05) higher H:L ratio. These observations are in consonance with the findings of Gross and Siegel (1983, 1986); Kuan *et al.* (1990); Craverner *et al.* (1992); Maxwell *et al.* (1992); Mitchell *et al.* (1992); Prabhakaran *et al.* (1997) and Puvadolpirod and Thaxton (2000a, c) under various stress conditions. However, Patterson and Siegel (1998) reported no significant change in percentage of heterophils but a change in lymphocyte count in birds subjected to increased cage density. Maxwell (1993) had comprehensively reviewed the reliability of H:L ratio as a biological indicator of stress in avian species.

Stress may cause involution of lymphoid tissues (thymus spleen and bursa of fabricius) which reduced the number of circulating lymphocytes and increased the number of heterophillic granulocytes (Garren and Shaffner, 1956; Glick, 1967; Siegel 1980, 1983). Direct lysis or apoptosis (Munck and Guyre, 1991) and delay in maturation (Sapolsky, 1992) of lymphocytes due to stress induced increase in corticosteroids may further exacerbate the problem of reduced number of lymphocytes in the circulation. Shapiro and Schechtman (1949) reported that a single injection of adrenocortical extract in adult fowl caused lymphocytolysis leading to transient lymphopenia.

Gross and Siegel (1983) stated that H:L ratio was more reliable indicator of perceived magnitude of stress than the plasma corticosterone values in avian species.

The present study highlighted the magnitude of variation in lymphocyte and heterophil counts and H:L ratio at eighth week was of lesser degree when compared to sixth week of age. There appeared a degree of adaptation upon continuous imposition of a stressor for prolonged period. This observation is in close agreement with earlier reports of Nir *et al.* (1975), Katanbaf *et al.* (1989), Maxwell *et al.* (1992) and Zulkifli *et al.* (1993). Evidence are accumulating to show that chicken readily habituate to fasts of moderate duration (Freeman *et al.*, 1981; Rees *et al.*,1985; Gross and Siegel, 1986; Zulkifli *et al.*, 1993).

Dietary supplementation of Zeetress® and ascorbic acid significantly ($P<0.05$) improved lymphocyte count, decreased heterophil count and H:L ratio in birds of both floor space reduced groups (G-III and G-IV respectively) and feed restricted groups (G-VI and G-VII respectively) indicating the antistress activity of Zeetress® and ascorbic acid.

Chicken synthesize ascorbic acid primarily in kidney (Roy and Guha, 1958). The quantity synthesized was supposed to be sufficient for normal growth and metabolism (Sealock and Goodland, 1951). But under stressful circumstances synthesized ascorbic acid may not meet physiological needs (Freeman, 1971; Siegel, 1971; Hornig *et al.*, 1984). Ascorbic acid could reduce the synthesis of glucocorticoids in birds under stress (Pardue and Thaxton, 1986) as plasma corticosteroid levels were significantly greater for heat stressed nonsupplemented chicks than for ascorbic acid supplemented heat stressed chicken (Pardue *et al.*, 1985).

Bhattacharya and Ghosal (1994) reported about the antistress activity of Zeetress® in rats and ascertained that Zeetress® was associated with attenuation of stress induced alterations in adrenal gland weight, levels of adrenal ascorbic acid and corticosterone and plasma corticosterone which indicated the evidence of suppression of hypothalamo-pituitary adrenocortical (HPA) activity induced by stress. They also pointed out that Zeetress® did not exert any discernible antistress activity on single large dose but was able to attenuate stress following prolonged smaller doses for seven days.

The number of monocyte and eosinophil in birds of floor space reduced group G-II and feed restricted group G-V did not vary significantly with that of control group G-I both at sixth and eighth week of age this observation was in accordance with the findings of Maxwell *et al.* (1990a) and Maxwell (1993) who reported no significant differences in monocyte and eosinophil counts between feed restricted and *ad libitum* fed birds. However, Mcfarlane *et al.* (1989) reported that monocyte was the only leucocyte to have a significant increase in number when birds were subjected to a continuous sound stress (80-95 decibels). The dietary Zeetress® and ascorbic acid supplementation in birds of floor space reduced groups (G-III and G-IV) and feedrestricted groups (G-VI and G-VII respectively) did not show any significant influence on either monocyte or eosinophil counts both at sixth and eighth week of age.

The basophil count in birds of both floor space reduced group G-II and feed restricted group G-V without any dietary antistress agent was significantly ($P<0.05$) higher when compared to that of control group G-I, indicating the increase in basophil number was associated with stress response. This observation closely agreed with the findings of Maxwell *et al.* (1990a, 1991, 1992), Maxwell (1993) and Maxwell and Robertson (1995). Maxwell *et al.* (1992) reported that feed restricted birds showed an increase in basophil number and they opined that heterophilia might be a response to mild to moderate stress whereas basophilia might be the response of severe stress. Dietary Zeetress® and ascorbic acid supplementation significantly ($P<0.05$) lowered the basophil count in birds of both floor space reduced groups (G-III and G-IV) and feed restricted groups (G-VI and G-VII respectively) when compared to respective groups G-II and G-V both at sixth and eight week of age with the exception of ascorbic supplemented group G-VII at sixth week of age where basophil count was lower but insignificant. These findings indicated the antistress activity of Zeetress® and ascorbic acid.

5.1.7 Mitogen induced lymphocyte blastogenic response.

Proliferative responses of lymphocytes to Concanavalin A (Con A) and Lipoplysaccharide (LPS) mitogen had been suggested as a measurement of chicken lymphocyte proliferation (Toivanen and Toivanen, 1973; Hovi *et al.*, 1978 and Haq *et al.*, 1996). In the present study the birds of floor space reduced group G-II and feed restricted group G-V considerably suppressed the mitogen induced lymphoblastogenic response when compared to that of the birds in control group G-I both at sixth and eighth week of age. This observation was in support of Frank Bleecha *et al.* (1984) who reported that elevated glucocorticoids in stressed animals were responsible to suppress the production of interleukin-2 by lymphocytes which was an essential proliferative factor, impairing the lymphocyte blastogenic response. Heat stress in birds stimulated the release of catecholamines and corticosterone and initiates peroxidation in cell membranes including membranes of T and B lymphocytes (Freeman and Crapo, 1982).

Dietary supplementation of Zeetress® and ascorbic acid improved the lymphoproliferation in response to mitogen in birds of both floor space reduced groups (G-III and G-IV) and feed restricted groups (G-VI and G-VII respectively) at sixth and eighth week of age when compared to their respective floor space reduced group G-II and feed restricted group G-V, suggesting the antistress activity of Zeetress® and ascorbic acid. The observation was in close agreement with Johnston and Huang (1991) who reported that ascorbic acid in diet enhanced the lymphocyte proliferation by improving the responsiveness of T lymphocytes to mitogens.

Ascorbic acid had been demonstrated to enhance antioxidant activity of Vitamin E by reducing the tocopheroxyl radicals back to their active form of vitamin E (Jacob 1995) or by sparing the available vitamin E (Retsky and Frei, 1995). Corwin and Shloss (1980) reported that vitamin E enhanced lymphocyte blastogenic to mitogens by protecting the lymphocytes from lipid peroxidation by the antioxidant activity. Puthpongsiriporn *et al.* (2001) opined that hens supplemented with a combination of 65 IU vitamin E and 1000 ppm ascorbic acid per kg feed showed an additive improvement in invitro lymphocyte proliferation in response to Con A and LPS mitogens compared to vitamin E or ascorbic acid supplementation alone. Later in 1994, Das and Chatterjee observed that administration of Stresszee® (a product similar to Zeetress® for human use) in rats resulted in an increase of lymphocyte population dynamics with potentiated cellular and humoral response against pathogenic stimuli.

5.2 Effect of induced stress and antistress agents on biochemical parameters

5.2.1 Plasma protein profile

In the present study there was no significant variation in total proteins, albumin, globulin and Albumin:Globulin ratio (A:G) either in birds of floor space reduced group G-II or in feed restricted group G-V without any dietary antistress agent when compared to that of control birds (G-I) both at sixth and eighth week of age probably due to fact that floor space reduction and feed restriction being a chronic stressor or this may be due to level of floor space reduction or feed restriction imposed in this study was not severe enough to cause a marked variation in plasma protein profile (table 5a).

At sixth week of age dietary supplementation of Zeetress® and ascorbic acid has no significant ($P<0.05$) influence on total plasma protein, albumin, globulin concentration and A:G ratio either in birds of floor space reduced groups (G-III and G-IV) or in feed restricted groups (G-VI and G-VII) when compared to respective groups G-II and G-V.

At eighth week of age Zeetress® supplementation in birds of floor space reduced group G-III showed a significantly ($P<0.05$) lower albumin concentration when compared to G-II without any dietary antistress agent indicating that the Zeetress® is having an effect on protein metabolism in liver and had a role in formation of glucose from non carbohydrate sources to supply energy in stressed birds. The A:G ratio in ascorbic acid supplemented floor space reduced group G-IV and Zeetress® supplemented feed restricted group G-VI were significantly ($P<0.05$) lower when compared to respective groups G-I and G-V, this was due to fact that a corresponding lower albumin value and higher globulin value was observed in these groups during that period.

5.2.2 Plasma lipid profile

5.2.2.1 Plasma cholesterol

Plasma cholesterol in birds of floor space reduced group G-II did not vary significantly ($P>0.05$) with that of control group (G-I) both at fourth, sixth and eighth week of age. This observation was in consonance with Gill and Sharma (1992) who reported that cholesterol level did not differ significantly between stocking densities (0.75 and 1 sq.ft/bird). Later Cetin and Tuncel (1995) also opined that housing density has no effect on plasma cholesterol value of 35 day old birds but in older birds (42 or 51 days old) an increase was observed when housed at 22 chicks/sq.m. This indicated that the level of floor space reduction induced in the present study was not severe enough to cause a significant variation in plasma cholesterol values.

Sayers *et al.* (1945) suggested that elevation of blood cholesterol level was one of the biochemical marker of stress response. Dietary supplementation of Zeetress® and ascorbic acid in floor space reduced groups G-III and G-IV significantly ($P<0.05$) lowered plasma cholesterol level at sixth week of age but the decrease was non significant at eighth week of age indicating the antiatherogenic property of these antistress agents. This observation was in support of Geetha (1993) who reported that stress induced hypercholestrolemia in rats was significantly attenuated by Stresszee® (a product similar to Zeetress® for human use).

The plasma cholesterol level in birds of feed restricted group G-V was significantly ($P<0.05$) lower at eighth week of age and at sixth week of age it was lower but non significant when compared to cholesterol level of control group (G I). This observation was in close agreement with findings of Zulkifli *et al.* (2000b) who observed a lower cholesterol concentration in feed restricted birds when compared to those fed *ad libitum*. Dietary Zeetress® and ascorbic acid supplementation in birds of feed restricted groups (G-VI and G-VII) showed no significant ($P>0.05$) influence on plasma cholesterol values both at sixth and eighth week of age. Among the periods comparatively higher cholesterol values were observed at sixth week of age when compared to eighth week of age. This observation was closely agreed with findings of Gill and Sharma (1992) who reported the sixth week cholesterol values in broiler chicken were higher compared to that of eighth week values indicating habituation with periods/growth.

5.2.2.2 Plasma triglycerides

Plasma triglycerides value of birds in floor space reduced group G-II did not vary significantly from that of control group G-I at fourth, sixth and eighth week of age indicating higher deposition of fat into fat depots which may be due to reduced activity of birds in floor space reduced groups. There was no significant ($P>0.05$) influence of dietary Zeetress® and ascorbic acid supplementation in birds of floor space reduced groups G-III and G-IV at sixth and eighth week of age when compared to G-II. No similar reports on plasma triglyceride levels in floor space reduced birds were available for comparison (table 5b).

In the present study the birds of feed restricted group G-V had significantly ($P<0.05$) lower plasma triglycerides concentration when compared to that of control group G-I both at sixth and eighth week of age. This observation is in consonance with findings of Bacon (1986), Anthony *et al.* (1990), Hocking *et al.* (1994) and Huff *et al.* (1996). However, Nir *et al.* (1973) reported that plasma triglycerides was not affected by starvation in cockerels and geese. In broiler chicken of feed restricted groups dietary supplementation of Zeetress® (G-VI) and ascorbic acid (G-VII) significantly ($P<0.05$) improved plasma trigycerides value both at sixth and eighth week of age indicating the antistress activity of Zeetress® and ascorbic acid in feed restriction stress. This observation is in consonance with findings of Mc Kee *et al.* (1997) who reported that plasma triglycerdies remained elevated in feed restricted birds supplemented with ascorbic acid and they concluded that ascorbic acid supplement influenced body energy stores that were used for energy purpose during periods of reduced energy intake.

5.2.2.3 Plasma total lipids

The plasma concentration of total lipids in bird of floor space reduced group G II was significantly ($P<0.05$) lower at sixth week of age where as it did not vary significantly ($P<0.05$) at eighth week of age when compared to that of birds of control group G-I. However, Cetin and Tuncel (1995) reported that the housing density had no effect on total lipids value of 35 day old birds but increased total lipids value was observed in older birds (42 or 52 days old) when housed at 22 chicks/sq.m and they concluded the stress symptoms could occur in chicks housed in groups of 22 chicks/sq.m and above. The present study indicated that the level of induced floor space reduction stress was not severe enough in broiler chicken to cause a marked variation in total lipids values.

In birds of feed restricted group G-V the total lipids value was significantly ($P<0.05$) lower at sixth week of age but non significant ($P>0.05$) at eighth week of age when compared to that of control birds in group G-I and was in consonance with findings of Katanbaf *et al.* (1989).

Bacon (1986), Anthony *et al.* (1990) and Vanderwal *et al.* (1999) reported that there is significant elevation of plasma free fatty acids (FFA) upon feed restriction or fasting. However, Bacon (1986), Hocking *et al.* (1994) and Huff *et al.* (1996) reported that a significant decrease in plasma triglycerides level occurred due to fasting or feed restriction in broiler chicken as triglycerides forms the major (59.7%) and FFA the minor (1.8%) component of plasma lipids in chicken (Christie and Moore, 1972), hence corresponding decrease in plasma triglycerides in fasting may lead to consequent decrease in total lipids value.

Dietary ascorbic acid supplementation significantly ($P<0.05$) lowered the value of total lipids in birds of floor space reduced group G-IV when compared to G-II at sixth week of age without any significant influence at eighth week of age. Dietary Zeetress® supplementation had no significant influence on total lipid value in birds of floor space reduced group G-III both at sixth and eighth week of age. The dietary Zeetress® and ascorbic acid supplementation in the birds of feed restricted groups (G-VI and G-VII respectively) had no significant ($P>0.05$) influence on plasma total lipid value at sixth week of age. However, significantly increased plasma concentration of total lipids at eighth week of age was observed when compared to that of G-V (table 5b) indicating that Zeetress® and ascorbic acid had the antistress activity in broiler chicken.

5.2.3 Plasma glucose

There was no significant variation in plasma glucose concentration in birds of floor space reduced group G-II without any dietary antistress agent from that of control group G-I (table 5c). This observation was not in agreement with findings of Cetin and Tuncel (1995) who reported an increased blood glucose level in 35 day old broilers when housing density was increased (22 birds/m^2). Latour *et al.* (1996) and Puvadolpirod and Thaxton (2000a) reported that continuous infusion of ACTH increased plasma glucose in chicken. In the present study there might have been a slight rise in plasma glucose in the initial periods due to stress response and then restored to control levels as the period of floor space reduction was prolonged or it may be due to the fact that floor space reduction being the chronic stressor failed to show any discernible effect on plasma glucose concentration. Nir *et al.* (1975) reported that plasma glucose concentration was markedly increased by ACTH administration after one hour and was almost restored to the control level after three hours.

In the birds of feed restricted group G-V there was slight but nonsignificant decrease in plasma glucose value when compared to that of control. This observation is in consonance with findings of Nir *et al.* (1973), Katanbaf *et al.* (1989) and Vanderwal *et al.* (1999).

Dietary supplementation of Zeetress® and ascorbic acid failed to show any significant influence on plasma glucose values in birds of both floor space reduced groups (G-III and G-IV) and feed restricted groups (G-VI and G-VII respectively) at sixth and eighth week of age (table 5c). Anthony *et al.* (1990) reported that overnight fasting in male turkeys did not produce any change in plasma glucose.

5.2.4 Plasma uric acid

Plasma uric acid in the present study showed significantly ($P<0.05$) lower values in the birds of floor space reduced group G-II at eighth week of age, whereas at sixth week of age it was lower but insignificant when compared to that of birds of control group G-I. In birds of feed restricted group G-V a significantly ($P<0.05$) lower uric acid concentration was observed both at sixth and eighth week of age (table 5c), which was in consonance with the findings of Sykes (1971) who observed that starvation significantly decreased the uric acid level both in blood and urine. Deyhim *et al.* (1995) and Guo and Liu (1997) reported that level of uric acid were significantly lower in birds under heat stress. Anthony *et al.* (1999) opined that the concentration of uric acid remained relatively low until the second day of feed withdrawal and thereafter it increased indicating that proteins were degraded to supply energy. In the present study the lower levels of uric acid suggested that 30% feed restriction was not severe as that of feed withdrawal or starvation and sufficient source of energy (carbohydrate and fats) was available in these birds.

In birds uric acid is synthesized in liver and it is filtered by glomeruli and secreted by kidney tubules. Uric acid is the chief nitrogenous end product of protein metabolism and urea that of purine metabolism in birds. Birds lack carbamyl phosphate synthetase necessary for synthesis of carbamyl phosphate which is a precursor for orinthine-urea cycle (Sturkie, 1976). In birds if diet is deficient in essential aminoacids, it catabolizes body protein to obtain these aminoacids. This process naturally led to an increased production and excretion of nitrogen mostly as uric acid.

Dietary Zeetress® supplementation improved the stress induced decrease in plasma uric acid in birds of both floor space reduced group G-III at sixth and eighth week of age and in feed restricted group G-VI at eighth week of age. Dietary ascorbic acid supplementation significantly ($P<0.05$) improved plasma uric acid in birds of floor space reduced group G-IV and feed restricted group G-VII only at eighth week of age, when compared to their respective groups G-II and G-V without any dietary antistress agents (table 5c).

5.2.5 Plasma Cortisol

Corticosteroid concentration in the blood has been used as a measure of environmental stress and physiological activity in chickens (Eskeland and Blom, 1979; Siegel, 1980). In birds corticosterone is the principle corticosteroid and cortisol is present in minute amounts.

In the present study significant increase ($P<0.05$) in the level of cortisol was observed in birds of floor space reduced group G-II and in feed restricted group G-V when compared to that of control group G-I at both sixth and eighth week of age indicating increased activity of adrenocortical response to stress. An increase in circulating level of corticosterone was observed in turkeys by Brown and Nestor, 1973 and EL-Halawani *et al.*, 1973 and in chicken by Edens and Siegel, 1975 and Zacharisen and Newcomer, 1975) shortly after an exposure to a stressor. Pesti and Howarth (1983) reported that plasma corticosterone were significantly higher for male broiler chicks housed on battery brooder kept at 697 and 348 cm^2/birds than when kept at 232 or 116 cm^2/bird. Bartov *et al.* (1988) stated that plasma corticosterone level was consistent and at times significantly ($P<0.05$) higher in fasted birds. However, Gill and Sharma (1992) were of opinion that cortisol level in broiler chicken did not differ significantly between stocking densities (0.75 and 1 sq.ft/bird). In the present study a floor space of 0.525 sq. ft/bird (492 sq.cm/bird) was provided for birds of G-II group which is comparatively higher stocking density than that of Gill and Sharma (1992) to cause a sufficient stress response.

Antistress activity of Zeetress® was well narrated by Bhattacharya and Ghosal (1994), Geetha (1993), Wheeler (1993) and Das (1994). In the present study dietary supplementation of ascorbic acid in broiler chicken of feed restricted group (G-VI and G-VII) significantly ($P<0.05$) lowered the plasma cortisol value at sixth week of age when compared to G-V without any dietary antistress agent but the decrease in cortisol value was not significant at eighth week of age. In birds of floor space reduced groups dietary Zeetress® (G-III) and ascorbic acid (G-IV) supplementation showed lower (insignificant) cortisol values both at sixth and eighth week of age, indicating antistress activity of both ascorbic acid and Zeetress®. Degkwitz (1987) reported that ascorbic acid supplementation in guinea pigs lowered the blood levels of cortisol.

Ascorbic acid could reduce the synthesis of glucocorticoids under stress conditions (Pardue and Thaxton 1986) as plasma corticosteroid levels are significantly greater for heat stressed non supplemented chicken than for ascorbic acid supplemented heat stressed birds (Pardue *et al.*, 1985). Bhattacharya and Ghosal (1994) reported that Zeetress® reversed the stress induced rise in plasma corticosterone and depletion of adrenocortical ascorbic acid brought about by augmented synthesis and release of adrenocorticotropin (ACTH). Nadig *et al.* (2002) reported that Stenot® (a product with similar composition as that the Zeetress®) lowered the plasma corticosterone in floor space reduced rats.

In the present study, with the exception of birds of control group G-I all the floor space reduced and feed restricted groups showed a comparatively higher cortisol values at sixth week of age than eighth week values indicating increased resistance to stress with age/growth. This observation is in consonance with findings of Gill and Sharma (1992). EL-Halawani *et al.* (1973) had traced the course of acclimatization to change in environmental temperature and reported that plasma corticosterone concentration returned to its initial concentration after two to three weeks depending on the intensity of stressor.

5.3 Effect of induced stress and antistress agents on production parameters

5.3.1 Mortality

No mortality was recorded in present study either in birds of floor space reduced groups (G-II, G-III and G-IV) or in feed restricted groups (G-V, G-VI and G-VII). This observation was in accordance with findings of Deaton *et al.* (1968) who opined that stocking density did not significantly affect the mortality percentage. However, Sheriff and Kothandaraman (1987) observed higher mortalities with increasing stocking densities. Imaeda (2000) found that sudden death syndrome (SDS) mortality increased at a stocking density of 18 birds/sq.m. Low mortality rate have been reported in heat stressed birds fed with supplemental ascorbic acid (Ahmad *et al.*, 1967, Gross, 1988, Abd-Ellah, 1995 and Jaffar and Blaha, 1996). However, no improvement in survivability of birds was observed when ascorbic acid was supplemented @ 100 mg/kg feed (Freeman *et al.*, 1983; Pardue *et al.*, 1985).

5.3.2 Body weight and weekly body weight gain

In the present study there was no significant ($P<0.05$) variation in body weight and weekly body weight gain between the birds of floor space reduced group G-II and that of control group (G-I) during the entire period of experiment (table 6a). This observation was in consonance with findings of Siegel and Coles (1958), Sulane and Ledaiyc (1976), Mandlekar and Thatte (1986) and Alribdawi and Singh (1989). However, Bhargava *et al.* (1975), Zoog (1980), Quinones *et al.* (1987) and Patterson and Siegel (1998) reported reduced body weight and weekly weight gain at higher stocking densities when compared to birds at lower stocking densities.

Dietary Zeetress® supplementation in birds of floor space reduced group G-III had no significant (P<0.05) influence on body weight and weight gain on all weeks except at eighth week of age it showed a significantly lower weekly body weight gain when compared to G-II. It showed superior body weight at fifth, sixth and seventh week of age and superior weight gains at fifth and sixth week of age.

Dietary ascorbic acid supplementation in birds of floor space reduced group G-IV had no significant influence on body weight and weekly body weight gain at fifth, sixth and seventh week of age but had significantly (P<0.05) lower weekly body weight gain at eighth week of age when compared to that of birds of G-II group. There was higher body weight and weight gain at fifth to seventh week of age. The lower weight gain at eighth week of age may be due the extra shortage of floor space induced by increased growth, size and bodyweights in these groups upto seventh week of age thus intensifying the floor space reduction stress or the higher dosage of these antistress agents might have proved beneficial at eighth week of age. The higher final body weight in birds of floor space reduced groups (G-II and G-III) may be due reduced activity in these birds because of low floor space availability which might have led to decreased maintenance requirement with increased storage of nutrients.

In the present study the body weight at fifth, sixth, seventh and eighth week and weekly weight gain at fifth, sixth and seventh week of age were significantly ($P<0.05$) lower in birds of feed restricted group G-V without any dietary antistress agent when compared to that of control group G-I. These observations are in consonance with findings of Nir *et al.* (1975), Katanbaf *et al.* (1988), Fontana *et al.* (1992) and Zulkifli *et al.* (1993).

Dietary supplementation of Zeetress® in birds of feed restricted group G-VI showed superior body weight and weekly body weight gain at almost all weeks of age when compared to that of G-V group indicating the antistress activity of Zeetress®. This observation was in support of Wheeler (1993) and Das (1994) who pointed out that Zeetress® ameliorated various undesirable effects of stress. Wheeler (1994) reported that Zeetress® treated broiler chicken exhibited increased body weight gain. Dietary ascorbic acid supplementation in birds of feed restricted group G-VII improved the body weight and weekly body weight gain when compared to birds of G-V at almost all weeks of age indicating ascorbic acid had the antistress activity. This was supported by Thaxton and Pardue (1984), Kutlu and Forbes (1993) and Mohammed (1997) whose reports were supplemental ascorbic acid reduced the weight losses due to stress, improved poultry performance and improved body weight of chicken, respectively.

5.3.3 Feed Efficiency

In the present study feed efficiency was better at fifth and sixth week of age of broiler chicken whereas poor feed efficiency was recorded at seventh and eighth week of age in almost all the groups (table 6b). By taking overall feed efficiency into consideration the poorest feed efficiency was for the birds of feed restricted group G-V without any dietary antistress agent. Poor feed efficiency in these feed restricted birds might be due to increased competition among birds for limited feed supply resulting into a psychological stress response further leading to weight loss and poor feed efficiency. However, Fontana *et al.* (1992) and Zulkifli *et al.* (1993) reported a significantly better feed conversion efficiency in feed restricted birds.

The birds of floor space reduced group G-II without any dietary antistress agent had comparatively better overall feed efficiency when compared to that of control group G-I. This observation was in consonance with findings of Bolton *et al.* (1972), Sheriff and Kothandaraman (1987), Shanaway (1988), Kuan *et al.* (1990), Narayanankutty and Ramakrishnan (1992) and Patterson and Siegel (1998). However, no significant change in feed efficiency with higher stocking densities was reported by Siegel and Coles (1958), Gill and Sharma (1992), Cravener *et al.* (1992) and Imaeda (2000).

Dietary supplementation of Zeetress® considerably improved the overall feed efficiency in birds of both floor space reduced group G-III and feed restricted group G-VI when compared to their respective groups G-II and G-V, indicating the antistress activity of Zeetress®. This observation was in support of findings of Wheeler (1994).

Ascorbic acid supplementation in diet did not proved beneficial in birds of floor space reduced group G-IV when compared to G-II, whereas in the birds of feed restricted group G-VII it considerably improved overall feed efficiency when compared to G-V. This indicated the better antistress activity of ascorbic acid in feed restriction stress. In chicken under heat stress supplemented with ascorbic acid improved growth performance when compared to birds of induced stress unsupplemented with any antistress agents (Thaxton and Pardue, 1984; Kutlu and Forbes, 1993; Abd-Ellah, 1995; McKee and Harrison, 1995 and Mohammed, 1997).

Summary

6. SUMMARY

The study was undertaken with the objective of evaluating the influence of induced stress and stress ameliorating effects of dietary supplementation of ascorbic acid and commercially available antistress agent - Zeetress® in broiler chicken by estimating certain physiological, biochemical and production parameters. Fifty six numbers of day old broiler chicks (Vencob) procured from a commercial hatchery, reared under standard managemental condition in battery cages. They were randomly selected and divided into seven groups (G-I to G-VII) with eight birds in each group. The birds in various groups were as follows: G-I as control (734 sq.cm/bird), G-II as floor space reduced group (33% reduction in floor space i.e., 492 sq.cm/bird), G-III as floor space reduced group supplemented with Zeetress® @ 0.01%, G-IV as floor space reduced group supplemented with ascorbic acid @ 0.02%, G-V as 30% feed restricted group, G-VI as 30% feed restricted group with dietary supplementation of Zeetress @ 0.01%, G-VII as 30% feed restricted group with dietary supplementation of ascorbic acid @ 0.02%. Production parameters such as body weight, weekly weight gain, feed consumption and feed efficiency were analyzed on weekly basis. Blood was collected with suitable anticoagulants, at fourth, sixth and eighth week of age. Analysis of haematological parameters such as total erythrocyte count (TEC), haemoglobin (Hb), volume of packed red cells (VPRC), erythrocyte sedimentation rate (ESR), total leucocyte count (TLC), erythrocyte indices, differential leucocyte count (DLC), heterophil: lymphocyte

(H:L) ratio and mitogen induced lymphocyte proliferation test. Plasma was utilized for biochemical analysis viz., plasma protein profile (total plasma protein, albumin, globulin, calculation of albumin:globulin (A:G) ratio), plasma lipid profile (cholesterol, triglycerides, total lipids), glucose, uric acid and cortisol at fourth, sixth and eighth week of age. The data were anlaysed statistically.

There was no variation in values of TEC, Hb, VPRC, ESR, TLC, MCV, MCH and MCHC of birds in G-II group when compared with control birds in G-I group. However, the birds of G-V group showed lower value of TEC, Hb, VPRC and TLC both at sixth and eight week of age. The dietary supplementation of Zeetress® and ascorbic acid in floor space reduced groups (G-III and G-IV) feed restricted groups (G-VI and G-VII) did not show any significant influence on values of TEC, Hb, VPRC, ESR, TLC when compared to birds of respective groups in G-II and G-V.

Lower lymphocyte count and higher heterophil and basophil count, higher H:L ratio and suppressed mitogen induced lymphocyte proliferation (probably due to the rise in plasma corticosteroids as a result of stress response) were recorded in birds of G-II and G-V group when compared to G-I group, both at sixth and eighth week of age. Dietary supplementation of Zeetress® and ascorbic acid in floor space reduced groups and feed restricted groups increased the lymphocyte count, lowered heterophil and basophil count, lowered H:L ratio and improved mitogen induced lymphocyte proliferation both at sixth and eighth week of age when compared to birds of respective groups G-II and G-V.

There was no significant variation in plasma concentration of glucose and protein profile of birds of G-II and G-V groups compared with control birds of G-I group both at sixth and eighth week of age. In birds of G-II and G-V groups the plasma cortisol concentration was higher both at sixth and eighth week of age when compared to that of birds of G-I group indicating a stress induced hyperactivity of adrenals. Dietary supplementation of Zeetress® and ascorbic acid in floor space reduced groups and in feed restricted groups showed a lower cortisol value when compared to birds of their respective groups G-II and G-V indicating their antistress activity.

The plasma uric acid concentration in birds of G-II and G-V groups was lower at sixth and eighth week of age when compared to control birds of G-I group. Dietary Zeetres® supplementation in birds of G-III and G-VI groups increased the uric acid concentration when compared to birds of G-II group both at sixth and eighth week of age. However, ascorbic acid supplementation in G-IV and G-VII groups significantly increased uric acid concentration only at eighth week of age.

No significant variation was observed in plasma lipid profile of birds in G-II group when compared to control birds of G-I group both at sixth and eighth week of age except a lower total lipid value at sixth week of age. In birds of G-V group plasma lipid profile was lower when compared to birds of G-I group. The dietary Zeetress® and ascorbic acid supplementation in G-VI and G-VII increased triglyceride concentration both at sixth and eighth week of age, with increased total lipid concentration at eighth week of age, when compared to values of birds in G-V group.

Body weight and weight gain in birds in floor space reduced groups (G-II, G-III and G-IV) did not vary from that of birds in control group G-I at all week of age, however, in birds of feed restricted groups (G-V, G-VI and G-VI) a lower body weight and weight gain was noted when compared to birds of control group (G-I), at all weeks of age.

Considering the overall feed efficiency the birds of G-II group had better feed efficiency (2.26) than that of control birds of G-I group (2.29) and Zeetress® supplementation in birds of G-III group showed slightly better feed efficiency (2.25) whereas ascorbic acid supplementation in G-IV group could not improve the overall feed efficiency (2.37) but had better feed efficiency upto seventh week of age, when compared to birds of G-II group. Considering the overall feed efficiency of birds in G-V group. They had overall poor feed efficiency (2.59) than that of birds in G-I group (2.29). Dietary supplementation of Zeetress® and ascorbic acid in birds of G-VI and G-VII groups improved the feed efficiency (2.22 and 2.35 respectively) in comparison with birds of G-V group.

The observations of the study revealed that 33% floor space reduction and 30% feed restriction induced certain level of chronic stress in broiler chicken resulting in variation of haematological and biochemical parameters with impaired growth especially in feed restricted group. Analysis of TLC, DLC, mitogen induced lymphoblastogenic response and plasma cortisol values indicated that there was a marked level of immunosuppression in the stress induced broiler chicken. The magnitude of variation in most of these parameters was higher at sixth week of age when compared to that of eighth week values indicating some sort of habituation in the broiler chicken was observed upon chronic stress. The antistress agents – Zeetress® and ascorbic acid were equally effective in reversal of stress induced alterations in haematological, biochemical parameters but in case of production parameters the Zeetress® had proved comparatively more efficient than ascorbic acid.

Further studies are required to bring pronounced effect of induced stress and antistress agents by increasing the degree of floor space reduction and feed restriction and by increasing the level of antistress agents in broiler chicken.

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EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON THE PHYSIOLOGICAL PARAMETERS IN BROILER CHICKEN

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ABSTRACT

In modern poultry production system various factors namely floor space reduction, feed and water restriction, high environmental temperature, vaccination, medication, debeaking etc. have the potential to induce stress in poultry which results in poor performance as well as reduction in immune response as the nutrients normally utilized for growth and production are diverted to counteract the stress and survival. The study was undertaken with the objective of evaluating the influence of induced stress and antistress agents (dietary ascorbic acid and Zeetress®) on various physiological, biochemical and production parameters in broiler chicken. Fifty six numbers of day old broiler chicks (Vencob) procured from a commercial hatchery were reared in battery cages under the standard managemental conditions upto four weeks of age. Then they were randomly selected and divided into seven groups (G-I to G-VII) with eight birds per group. The birds in different groups were as follows: G-I as the control, G-II as floor space reduced group (33%), G-III as floor space reduced group supplemented with Zeetress® @ 0.01%, G-IV as floor space reduced group supplemented with ascorbic acid @ 0.02%, G-V as 30% feed restriction, G-VI as 30% feed restriction with dietary supplementation of Zeetress® @ 0.01% and G-VII as 30% feed restriction with dietary supplementation of ascorbic acid @ 0.02%. The birds were maintained in battery cages under standard managemental conditions on broiler ration with the exception of floor space reduction, feed restriction and incorporation of dietary antistress agents.

Production parameters such as weekly weight gain and feed efficiency were analyzed on weekly basis. Blood was collected with suitable anticoagulants, initially at fourth and then at sixth and eighth week of age. Whole blood was utilized for analysis of haematological parameters, heterophil: lymphocyte (H:L) ratio and mitogen induced lymphocyte proliferation test. Plasma was utilized for biochemical analyses i.e. for protein profile, lipid profile and estimation of glucose, uric acid and cortisol. The data were statistically analysed by appropriate tests.

The birds of G-V group showed significantly lower values of TEC, Hb, VPRC and TLC at sixth and eight week of age. The dietary supplementation of Zeetress® and ascorbic acid in floor space reduced groups feed restricted groups did not show any significant influence on the values of TEC, Hb, VPRC, ESR, TLC when compared to the birds of respective stress induced groups G-II and G-V.

The birds of stress induced groups G-II and G-V had lower lymphocyte count and higher heterophil and basophil count, higher H:L ratio and suppressed mitogen induced lymphocyte proliferation (probably due to a rise in level of plasma corticosteroids as a result of stress response) when compared to birds of G-I group, both at sixth and eighth week of age. 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 both at sixth and eighth week of age.

In the present study the birds of G-II and G-V group had no significant variation in plasma protein profile and glucose levels but had increased plasma concentration of cortisol (probably due to stress induced adrenal hyper activity) and decreased uric acid, when compared to birds of control group G-I, both at sixth and eighth week of age. Dietary Zeetress® and ascorbic acid supplementation in floor space reduced groups and feed restricted groups showed a lower plasma concentration of cortisol both at sixth and eighth week of age, and significantly increased uric acid at eighth week of age.

Dietary supplementation of Zeetress® and ascorbic acid in floor space reduced groups decreased plasma concentration of cholesterol both at sixth and eighth week of age and total lipids at sixth week of age. In birds of feed restricted G-V group plasma concentration of cholesterol, triglycerides and total lipids were lower both at sixth and eighth week of age when compared to birds of control group G-I. The dietary supplementation of Zeetress® and ascorbic acid in feed restricted groups had no significant influence on plasma concentration of cholesterol both at sixth and eighth week of age. However, there was an increased concentration of triglycerides both at sixth and eighth week of age with significant increase in total lipids at eighth week of age, when compared to birds of G-V group.

There was no significant variation in body weight and weight gain of birds in all floor space reduced groups compared to birds of control group (G-I) at all weeks of age with the exception of lower weight gain in G-III and G-IV when compared to G-II at eighth week of age. The body weight and weight gain in the birds of all feed restricted groups were lower when compared to birds of control group (G-I), at all weeks of age.

A perusal of the overall feed efficiency from 5 to 8 weeks of age revealed that the birds of G-VI group supplemented with Zeetress® recorded a superior FE (2.22) whereas an inferior value of 2.59 was noted in G-V group without any dietary antistress agent. The control group recorded a FE of 2.29. Zeetress® supplementation improved overall FE in feed restricted group (G-VI). On the other hand this advantage was not observed in floor space reduced group (G-III). Dietary ascorbic acid improved overall FE only in case of feed restricted group G-VII when compared to that of G-V group. However, in floor space reduced group (G-IV), ascorbic acid supplementation could not improve the FE.

The observations of the present study revealed that both feed restriction and floor space reduction resulted in some sort of chronic stress which led to variation in haematological and biochemical parameters, and impaired growth (in feed restricted groups). Analysis of TLC, DLC, mitogen induced lymphocyte blastogenic response and plasma cortisol values indicated that there was a marked level of immunosuppression in the stressed birds. The magnitude of variation in most of these parameters was higher at sixth week of age when compared to that of eighth week values indicating some sort of habituation/ adaptation occurred upon chronic stress in the broiler chicken. The antistress agents – Zeetress® and ascorbic acid were equally effective in reversal of stress induced alterations in haematological, biochemical and production parameters.