"EFFECT OF SEASON AND ANTISTRESS AGENTS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF BROILER CHICKEN UNDER STRESS"

By

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THESIS

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DECLARATION

I hereby declare that the thesis, entitled **"EFFECT OF SEASON AND ANTISTRESS AGENTS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF BROILER CHICKEN UNDER STRESS"** 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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CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	3
3	MATERIALS AND METHODS	41
4	RESULTS	60
5	DISCUSSION	154
6	SUMMARY	216
	REFERENCES	226
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Experimental groups of broiler chicken (n =12)	42
2	Composition of broiler chicken ration	45
3.1	Proximate principles of broiler chicken ration (On dry matter basis)	46
3.2	Proximate principles of broiler chicken ration (On dry matter basis)	47
4.1	Weekly meteorological data during the experimental period of eight weeks (Summer season)	61
4.2	Weekly meteorological data during the experimental period of eight weeks (Rainy Season)	62
5.1	Effect of overcrowding stress and antistress agents on haematological parameters of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	66
5.2	Effect of overcrowding stress and antistress agents on haematological parameters (Erythrocyte indices) of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	70
5.3	Effect of overcrowding stress and antistress agents on haematological parameters of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	75
5.4	Effect of overcrowding stress and antistress agents on haematological parameters (differential leukocyte count) of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	80
5.5	Effect of overcrowding stress and antistress agents on haematological parameters (differential leukocyte count) of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	86
6.1	Effect of overcrowding stress and antistress agents on plasma protein profile of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	90
6.2	Effect of overcrowding stress and antistress agents on plasma protein profile of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	93
7	Effect of overcrowding stress and antistress agents on plasma lipid profile of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	101

Table No.	Title	Page No.
8	Effect of overcrowding stress and antistress agents on plasma glucose concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	108
9	Effect of overcrowding stress and antistress agents on plasma enzymes of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)	121
10	Effect of overcrowding stress and antistress agents on weekly body weight of broiler.chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=24)	137
11	Effect of overcrowding stress and antistress agents on weekly body weight gain of broiler chicken from fifth to eighth week of age in summer and rainy seasons. Mean ± SE (n=24)	140
12	Effect of overcrowding stress and antistress agents on weekly feed intake of broiler chicken from fifth to eighth week of age in summer and rainy seasons. Mean ± SE (n=24)	143
13	Effect of overcrowding stress and antistress agents on cumulative feed efficiency of broiler chicken from fifth to eighth week of age in summer and rainy seasons. Mean ± SE (n=24)	145
14	Effect of overcrowding stress and antistress agents on organ weight of broiler chicken at eighth week of age in summer and rainy seasons. Mean ± SE (n=24)	151
15	Cost benefit analysis as influenced by overcrowding stress and antistress agents in eight week old broilers in summer and rainy seasons	153

Introduction

LIST OF FIGURES

Figure No.	Title	Page No.
1.1	Unstressed control group of broilers chicken (G-I)	43
1.2	Stressed control group of broiler chicken (G-II)	43
2	SDS Polyacrylamide Electrophoretogram of plasma proteins of broiler chicken under the influence of stress and antistress agents in summer and rainy seasons	98
3	Effect of overcrowding stress and antistress agents on plasma corticosterone concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=6)	110
4	Effect of overcrowding stress and antistress agents on plasma cortisol concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=6)	113
5	Effect of overcrowding stress and antistress agents on plasma Triiodothyronine concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=6)	115
6	Effect of overcrowding stress and antistress agents on plasma Thyroxine (T_4) concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=6)	119
7	Effect of overcrowding stress and antistress agents on plasma bilirubin concentration in broiler chicken from fourth to eighth week of age in summer and rainy seasons. (n=12)	128
8	Effect of overcrowding stress and antistress agents on lymphoproliferative response in broiler chicken from fourth to eighth week of age in summer and rainy seasons.	130
9	Radial immuno-diffusion method for quantification of IgG	132
10	Effect of overcrowding stress and antistress agents on concentration of Immunoglobulin G (IgG) in broiler chicken from fourth to eighth week of age in summer and rainy seasons.	132
11	Effect of overcrowding stress and antistress agents on dressing percentage of broiler chicken from fifth to eighth week of age in summer and rainy seasons. (n=24) .	148

Introduction

1. INTRODUCTION

India, especially the southern peninsula is well noted for its presence in poultry rearing, both in layer and broiler sectors. Interestingly, broiler industry in Indian Agriculture has taken a quantum leap over the last few decades with an annual growth rate to the tune of 16 to 18 per cent in broiler chicken production. Since its inception in early 1970s, broiler farming has gone up quite fast and now India stands fifth largest producer of poultry meat, accounting for 1.6 million metric tons of poultry meat per annum (FAO, 2005). Chicken meat has emerged as an important part of human diet and has found a place in the regular food pyramid because it faces no religious taboos. The tremendous increase in output and efficiency in broiler chicken production over last few decades has created welfare problems among these birds.

In an eagerness to reap more profit, the intensive managemental practices adopted by farmers have put the birds under severe stress. Overcrowding or high stocking density is one of the major stressors encountered under the intensive farming system. Besides, the artificially imposed stress due to mismanagement, a wide variety of spontaneous stressors such as environmental stressors (light or dark) and climatic stressors (cold or hot) adversely affect the broiler well-being. Chicken are said to be sentient with the capacity to feel pain and suffer mental distress. They have emotions such as frustrations, boredom and hunger, and therefore while trying to improve the profit of production it is necessary to observe the well-being of birds (Morton, 2003).

The intense selection for economic traits in concert with increased mechanisation and changes in husbandry practices may result in a variety of stress responses. But understanding of stress syndrome remains elusive and creates confusions and controversy. In poultry production, the term stress is commonly used to justify unexplained inferior productivity or pathological states. But Selye (1976), the grand master of stress research, stated that stress was neither always the nonspecific results of damage nor always bad. He used the term "Eustress" to denote good stress and "Distress" for bad stress. Brown (1974) indicated that certain amount of stress is necessary in ameliorating boredom and in heightening endocrine functions. But severe stress or 'distress' would adversely affect important economic parameters like growth rate, feed efficiency, immune response, fertility, hatchability and livability in birds. Thus the implication of stress to poultry production is such that the ultimate aim of successful husbandry is not to eliminate stress but to maintain it at an optimum level.

Reports on the adverse effects of distress on broiler growth have resulted in the commercial production of several antistress agents and growth promoters for use in poultry. Antibiotics, probiotics, herbal antistress agents, vitamins, enzymes and liver tonics are but a few of the commonly used agents in poultry industry. Unfortunately most of them are used by farmers quite empirically without much study about their effects.

Although studies on stress is voluminous on perusal of literature, reports on the lone as well as combined effects of stress due to overcrowding and season in broiler production are scarce. Besides, the research on the antistress effect of growth promoters is also scanty. As such considering the importance of stress response and its impact on poultry production, the present research was undertaken with the following objectives:

1. Assess the effect of overcrowding stress on the haemogram, blood biochemical parameters and immune status in broiler chicken.
2. Ascertain the level of stress in these birds by estimating certain hormones that are related to stress and growth.
3. Evaluate and compare the stress ameliorating effects of certain antistress agents as L - ascorbic acid, virginiamycin and probiotic.

Review of Literature

2. REVIEW OF LITERATURE

Commercial poultry producers suffer considerable economic loss due to various kinds of constraints such as management, disease, climatic or environmental conditions, and stress. The damaging effects of stress on the physiological and biochemical parameters of poultry has been given little attention. Both genetic selection and environmental pressure had resulted in an increased sensitivity of livestock and poultry to stressors. Moreover, stress remains unnoticed most of the time, producing severe internal damages leading to a negative impact on economic traits. Even the rapid growth of broiler chicken itself can act as a source of stress. Most of the experimental studies had been concerned with acute stress, while chronic multiple stresses, which are more likely to be encountered in intensive poultry farming, had received little attention. Researchers face difficulty in measuring the welfare of birds and animals because there is no simple measure of the welfare. Moreover, all kinds of stressors were believed to produce exactly the same physiological response in animal species (Hill, 1983).

2.1 STRESSORS IN POULTRY PRODUCTION

The characteristic of living beings is their ability to maintain physiological and psychological stability. Anything that disrupts stability or homoeostasis, such as handling, noise or disruption of social order is a stressor. The reaction of the organism to stressor is regarded as stress, which functions in compacting and accommodating the stressor and is generally positive and adaptive (Williams, 1984).

Wolff (1960) divided the stressors into two types: the first type being the physical stimuli such as extremes of temperature or corrosive chemicals, which acted directly and produced a predictable physical response and the second type are those, which exerted this influence by acting as a signal.

Stressors common to poultry production include extremes of temperature, disease, handling, beak trimming, vaccinations, overcrowding, inadequate ventilation and transportation (Curtis, 1983). According to Griffin (1989), the stressors could be classified into three categories as (a) environmental, which included heat, cold, transportation, noval sound, odour and taste (b) behavioural – overcrowding, hierarchical challenge, weaning and feed restriction and (c) psychological – capture of animals and physical restraint.

According to Kettlewell (1989), stressors could be mental, physical and mixed. Stress during catching, social group and transportation formed mental stressors and harvesting, loading and carrying inverted into crate were the physical stressors. Saxena (1997) divided stressors into two categories, viz. avoidable stress (overcrowding, social behaviour, toxins in feed, diseases, poor ventilation, starvation, rough and frequent handling) and unavoidable stress (vaccination, transportation, medication, debeaking, extreme weather, and high rate of growth).

Too little or too much stress is undesirable for an animal's well being. But some stress is considered as biologically unavoidable for maintaining normal functions (Brown, 1974). The challenge in poultry production is to assess the degree of stress and provides an appropriate optimum stress environment. But stress is a term, which is difficult to define, identify and quantify (Hill, 1983). The major problem in measuring stress is that there are no generally agreed measures or methods in the assessment of stress. Although production performances as well as physiological and behavioural techniques are some of the typical indices of measures of stress, still there are skeptical views regarding the validity of these criteria.

2.1.1 Environmental Stress

The environment can be defined as the combination of external conditions, which have an impact on animals, birds or humans. The external conditions such as extremes of weather and climate can affect poultry production. Temperature is a very important factor in the bird's microenvironment, which needs to be managed properly. If poultry house environmental temperature is allowed to exceed normal ranges, growth and production will be negatively affected, as it affects the body metabolism of birds. Squibb and Wogan (1960) reported that irreversible heat prostration resulted when a period of relatively mild climate is followed by an extremely hot period. High humidity along with high environmental temperature makes the bird more uncomfortable.

India is located in the northern hemisphere 8° 4" and 37° 6" north and longitude 68° 7" and 97° 25" east. The climate in this region is described as tropical monsoon type (Anon, 1975). The seasons in India have been classified (ICAR 1977) into winter (October to February), summer (March to June) and rainy (July to September). In South India, no distinct winter season prevails and there are two wet periods and a distinct dry season (Nair, 1973). When extremes of temperature are the major problem in north India, high temperature along with high humidity is the problem in south India.

2.1.2 Overcrowding Stress

One of the often-overlooked stressors that affects production efficiency and physiological responses of birds is social stress caused by overcrowding. It had been documented that productivity rate generally declined as population size increased so that space allowance per bird decreased (Tomhave and Seegar, 1945). Much concern had been expressed about the negative effects of overcrowding in floor-housed and caged flocks with respect to air quality, disease incidence and aggression. Overcrowding in both broilers and layers might lead to higher mortality and decreased growth and reproduction in individual birds although overall economic returns from the flock might be greater. Stocking density is also viewed as a welfare concern.

2.2 EFFECTS OF STRESS ON PHYSIOLOGICAL PARAMETERS

2.2.1 Haematological Parameters

2.2.1.1 Total Erythrocyte Count (TEC) and Erythrocyte Indices

Breusch (1928) mentioned about the high variability of cell counts in chicken and considered it as normal. Palmer and Biely (1935), Twisselmann (1939) and Sturkie (1965) reported the normal erythrocyte count in chicken. Literatures regarding the effect of stress on TEC and erythrocyte indices are scanty. Donkoh (1989) observed a reduction of TEC value in broilers exposed to 30° to 35°C.

Furlan *et al.* (1999) noted erythrocytopenia in heat-stressed chicken. Reddy (2003) reported erythrocytopenia in feed restricted broilers, whereas floor space reduction (33%) had no effect on the TEC. According to him, neither feed restriction nor floor space reduction could induce any change in the values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Maxwell *et al.* (1990b) observed that the values of MCV and MCH were reduced and TEC was increased significantly when broilers were fed with a restricted diet. The MCHC and reticulocyte count (RC) did not alter significantly. They opined that feed restricted birds had a tendency for microcytic normochromic anaemia. Maxwell *et al.* (1991) observed that seven week old birds showed significant reduction in erythrocyte count with a significant increase in MCV and MCH at an early age feed restriction indicating macrocytic normochromic anaemia. Zhou *et al.* (1998b) reported no light or dark differences on the value of TEC or MCV in feed and water restricted birds when compared to non-restricted group.

2.2.1.2 Haemoglobin (Hb) Concentration and Volume of Packed Red Blood Cells (VPRC)

Winter (1935) noted that the Hb content of blood increased significantly during the winter season. Hutson (1965) reported that the value of VPRC increased as birds were exposed to cold as he observed haematocrit value of 31.8 per cent at 8°C as against a haematocrit value of 29.6 per cent at 30°C. Deaton *et al.* (1978) observed a higher haematocrit and Hb levels in birds reared under cold environments (10°C and 15.6°C when compared to 21°C). Heat distress caused a reduction in the values of haematocrit and Hb in birds, which was associated with haemodilution, an adaptive response to heat stress (Darre and Harrison, 1987; Donkoh, 1989; Furlan *et al.*, 1999; Borges *et al.*, 2004).

Yahav *et al.* (1997) observed a significant reduction in packed cell volume (PCV) in five to eight weeks old broiler chicks exposed to higher temperature (30 and 35°C) while acute exposure of eight weeks old chicks to high temperature (35°C for 6h) did not have any significant effect on packed cell volume. Zhou *et al.* (1998a) found no significant difference in the percentage of haematocrit in broiler chicken between 25°, 30° and 35°C, but significant difference was reported between 20° and 30°C. According to Borges *et al.* (1999), heat stress increased VPRC and Hb concentration in birds, whereas Furlan *et al.* (1999) reported that acute heat stress was associated with a decrease in the values of VPRC. Contrary to this, Altan *et al.* (2000) found that haematocrit values were not affected by heat stress (38±1°C) in 35 days old broilers.

Study by Emre *et al.* (1991), revealed that population density range of 10, 14, 18 and 20 chicks/m^2 did not affect the VPRC and Hb value in broilers of 40 and 49 days of age. As per the reports of Reddy (2003), stocking density of 22 birds/m^2 did not produce a significant change in Hb or VPRC values in six and eight weeks old broilers.

Halliday *et al.* (1977) could not observe any significant change in haematocrit in six to eight weeks old broilers transported for 16 to 18h. Hugher *et al.* (1984) in their study with *Gallus domesticus* concluded that restriction of food for 48h increased the haematocrit by 30 per cent. However, Maxwell *et al.* (1990b) and Reddy (2003) observed lowered value of VPRC and Hb in birds under feed restriction.

2.3.1.3 Total Leukocyte Count

Emre *et al.* (1991) in their study on six week old birds kept at a population density of 18 or 22 birds/m^2 noted leukocytosis when compared to those kept at 10 or 14 birds/m^2. On the other hand Zhou *et al.* (1998b) and Reddy (2003) could not observe any significant difference in the leukocyte count in birds under floor space reduction stress and feed restriction stress.

Shapiro and Schechtman (1949) observed leukocytosis on a single injection of adrenocortical extract in adult fowl, whereas Siegel (1968) obtained leukopenia in broilers on treatment with adrenocorticotropic hormone (ACTH) and cortisol. Leukopenia was also reported in birds subjected to heat stress (Thaxton *et al.*, 1968; Nathan *et al.*, 1976; Heller *et al.*, 1979; Mashaly *et al.*, 2004). Siegel (1983) opined that leukopenia could be considered as an indicator of stress in birds. Even though, Grey *et al.* (1989) observed leukocytosis in birds treated with low doses (6.3 IU/kg/day for 4 days) of ACTH, and opined that leukocyte count was an inconsistent and unreliable indicator of stress.

According to Maxwell *et al.* (1990a) and Maxwell *et al.* (1991) feed restriction resulted in leukopenia. Lazarevic *et al.* (2000) reported reduced leukocyte count in birds subjected to sound stress. Munck and Guyre (1991) opined that induced stress in birds could cause direct lysis and delay in maturation (Sapolsky, 1992) of leukocytes.

2 2.1.4 Differential Leukocyte Count (DLC)

A single injection of adrenocortical extract produced lymphocytosis followed by a transient lymphopenia (Shapiro and Schechtman, 1949). Huble (1955) obtained eosinopenia on treatment of cockerels with ACTH. Hammant (1962) opined that DLC could be used as a stress indicator in birds. Mc Farlan *et al.* (1989) obtained only monocytosis when birds were subjected to continuous sound stress. Gray *et al.* (1989) in their study on the hormonal effects of haematological parameters as indicators of chronic stress in laying hens noticed a significant heterophilia, eosinophilia and basophilia in seven to eight months old laying hens after daily injection of ACTH (6.3 IU/kg/day for 4 days). They concluded that absolute heterophil counts were the most sensitive haematopoietic indicator of chronic stress whereas; leukocyte, basophil and eosinophil counts were inconsistent and unreliable indicators of stress response.

As per Maxwell *et al.* (1990a) chicken basophils were the only type of white blood cells (WBC) to increase significantly in number on stress associated with feed restriction. They further reported that early age feed restriction (6, 10 or 14 days) in birds produced basophilia and eosinopenia. Maxwell *et al.* (1992) and Maxwell (1993) opined that in poultry, heterophilia might be the response on mild to moderate stress, whereas basophilia might be the result of severe stress. This was because, heterophils clearly responded in the early phase of hormonal surge but this increase could not be physiologically sustained and the prolonged effect negated further recruitment of these cells (Maxwell, 1993).

In avian species, feed restriction resulted in monocytopenia (Hocking *et al.*, 1994) and basophilia (Maxwell and Robertson, 1995). According to Maxwell and Robertson (1995) heterophil: lymphocyte (H/L) ratio was a less variable indicator of avian stress. However, during extreme stress as in life threatening situations, heterophilia and basophilia often developed. Thus in some stress situations a biphasic cellular reaction might be present.

2.2.1.5 Heterophil / Lymphocyte (H/L) Ratio

A significant increase in H/L ratio was noted by Mahtre and Joshi (1993) in Vencob chicks brooded at 95 to 100°F when compared to those brooded at 90 to 95°F or 85 to 90°F. Many reports revealed that the heat stress increased H/L ratio in birds (Swenson and Reece, 1996; Borges *et al.*, 2004).

Kuan *et al.* (1990) reported that increased stocking density produced stress indicated by higher H/L ratio from the fourth week onwards. As per Emre *et al.* (1991), increase in population density from 10 and 14 chicks/m^2 to 18 and 22 chicks/m^2 increased the H/L ratio in 40 and 49 days old broiler chicks. However, Cravener *et al.* (1992) found that seven week old birds reared at a floor space of 0.09 and 0.11m^2/ bird had significantly higher H/L ratio than those reared at 0.05 and 0.07 m^2/ bird and according to Patterson and Siegel (1998), cage density had no significant effect on H/L ratio in broilers.

Gross and Siegel (1993) observed that H/L ratio of about 0.2, 0.5 and 0.8 characterised low, optimum and high levels of stress, respectively. They suggested that in birds, H/L ratio was a good measure of long-term stress (hours or weeks).

Hammant (1962) observed an increase in H/L ratio in mature Leghorn hens following handling stress after starvation for 40h. Later, Saleh and Jaksch (1977) reported the same result in 52 days old female chicken subjected to heat stress. Many scientists opined that H/L ratio was a more reliable indicator of stress in avian species (Gross and Siegel, 1983; Maxwell, 1993; Siegel, 1995).

Gross (1984) noted increased H/L ratio after a fasting of 48h, which continued for more than 72h. Birds subjected to feed deprivation for two days at one-week interval showed an increase in H/L ratio and the increase was more severe on the first fasting stress than the second (Gross and Sigel, 1986). Jones *et al.* (1988) observed increased H/L ratio in laying hens after implanting minipumps delivering corticosterone. According to Grey *et al.* (1989); H/L ratio was a less conclusive indicator of chronic stress in birds.

Studies by Gross (1990) showed that in four to eight weeks old chicken subjected to sound stress (104db) for 30s, the H/L ratio began to increase 18h after the sound stress, reaching a maximum value of 0.62 in 20h and returned to pre-stress value of 0.27 after 30h. Similarly, cold stress, feed deprivation stress and water deprivation stress increased H/L ratio in chicken (Zabudskii and Skutar, 1993). Guemene *et al.* (2001) could not observe any significant change in H/L ratio in male Mule ducks subjected to force-feeding.

2.2.2 Blood Biochemical Parameters

2.2.2.1 Plasma Protein Profile

2.2.2.1.1 Total Proteins

Hutson and Subhas (1969) reported low concentration of total plasma protein in birds maintained at 30°C when compared to those kept at 18°C. Ward and Peterson (1973) also observed a lowered plasma protein concentration in birds subjected to chronic heat stress of 33° to 35°C for four weeks, when compared to those at 18° to 22°C for the same period. The elevated corticosterone concentration in heat-stressed birds (30° and 35°C) reduced amino acid transport from liver, which resulted in a reduced protein synthesis and reduced plasma protein concentration (Donkoh, 1989; Chunyan *et al.*, 1998). Belay and Teeter (1996) observed a decrease in the serum protein and albumin concentration in seven week old broilers subjected to cycling temperature of 24 to 35°C. Reports of Berrong and Washburn (1998) showed that the plasma protein level of broiler chicks exposed to 38°C was significantly lower than that of birds reared at 21°C and 32°C.

In the experiments with chicks, Polonis (1982) observed no significant difference in total serum protein levels between ages (5 to 8 weeks), between sex and between temperatures (12° to 14°C and 24° to 26°C). However, increased serum albumin level was observed between temperatures of 12° to 14°C and 24° to 26°C. Zhou *et al.* (1998a) could not observe any significant difference in the concentration of total proteins in broiler chicken between 7°, 20°, 25°, 30° and 35°C. Reddy (2003) could not observe any significant difference in concentration of plasma total proteins in birds under overcrowding stress (492cm^2/ bird) or under feed restriction stress.

Siegel (1971) opined that stress increased concentration of circulating glucocorticoids, which in turn increased total plasma protein concentration. It is reported that addition of cortisol and corticosterone in chicken muscle cell cultures decreased protein degradation (Klasing and Jarrel, 1984). Wilkoski and Zeiba (1989) in their studies with 12 weeks old Japanese quails concluded that blood concentration of plasma total proteins was variable between lines and sex. Puvadolpirod and Thaxton (2000a) obtained an increased concentration of plasma total proteins (2.95g/dl as against 2.71g/dl in control birds) in birds following ACTH infusion (16 IU/kg body weight/day for 7 days).

2.2.2.1.2 Albumin, Globulin and Albumin/ Globulin (A/ G) Ratio

Increased serum albumin level was also reported by Polonis (1982) in five to eight weeks old chicks when exposed to 12° to 14°C and 24° to 26°C. Deyhim *et al.* (1995) found that heat stress reduced serum albumin concentration. Fenghua *et al.* (1997) observed a lowered A/G ratio in 350 days old laying hens exposed to 34.5°C for 10 to 14 days when compared to those exposed to 26°C.

Reddy (2003) could not observe any significant difference in the levels of albumin, globulin and A/G ratio in birds under overcrowding stress (492sq. cm/ bird) or feed restriction stress.

As the birds were transported for a distance of 16 to 18 km a significant rise in serum albumin content was noticed without any effect on serum globulin concentration. The mean albumin concentration increased from 1.08g/dl before transportation to 1.18g/dl after 16 to 18 km transportation. (Halliday *et al.*, 1977).

Spinu *et al.* (1992) reported that in broilers transferred from large runs, to be caged in pairs, the serum gamma globulin concentration was significantly reduced for the first 10 days but not in the following 16 days.

2.2.2.2 Plasma Lipid Profile

2.2.2.2.1 Plasma Total lipids

Freeman (1978) observed hypolipidemia in newly hatched chicks exposed to high temperature (40°C for 60 min). Studies by Cetin and Tuncel (1995) showed that stress symptoms indicated by increased amount of total plasma lipids could occur in birds of 42 or 52 days of age when housing density increased to 22 birds/m^2 or more. However, they could not observe any significant change in lipid concentration at a housing density of 22 birds/m^2 at 35 days of age. Reddy (2003) in his study on the effect of floor space reduction (492cm^2/bird) and feed restriction (30%) stress could not find any significant change in plasma lipid concentration in eight week old broilers but plasma lipid level significantly reduced at sixth week of age.

According to Freeman *et al.* (1984), transportation (224km) stimulated glucagon release, which in turn increased lipolysis and raised plasma non-esterified fatty acids (NEFA) concentration.

Stress due to starvation resulted in a reduction in the plasma free fatty acids (FFA) content (Langslow *et al.*, 1970). Bacon (1986) reported that short term (upto 44h) feed restriction in male turkeys resulted in nearly three-fold increase in FFA concentration and approximately 70 per cent reduction in plasma concentration of triglycerides at all ages. Similar observations were also reported by Anthony *et al.* (1990) in eight to nine weeks old broiler turkeys after over night fasting. As per Hocking *et al.* (1994), concentration of plasma triglycerides was more than 30 per cent higher in fat line female birds than lean line female birds, when fed *ad libitum* or restricted. It is suggested that, in turkeys, concentration of serum triglycerides was the most sensitive indicator to either feed or water withdrawal with reduction occurring at 16h after feed withdrawal (Huff *et al.*, 1996).

Corticosterone injection caused an increase in total lipid concentration of plasma (Nagra and Meyer, 1963). Rudas *et al.* (1972) reported that high doses of thyroxine reduced the serum lipid concentration in broilers. Latour *et al.* (1996) observed an increase in plasma level of triglycerides in broiler chicks on continuous infusion of ACTH (8 IU / kg body weight / day). Puvadolpirod and Thaxton (2000a) also noted an increased plasma concentration (115.13 ng/dl as against 77.38 ng/dl in control group) in broilers on continuous infusion of ACTH (16 IU/kg body weight/day for 7 days).

2.2.2.2.2 Plasma Total Cholesterol

Rise in blood cholesterol was suggested to be one of the biochemical markers of stress response (Sayers *et al.,* 1945). Reports revealed that heat stress produced hypercholesterolemia in newly hatched chicken (Freeman, 1978; Guo and Liu, 1997). Polonis (1982) did not observe any significant change in plasma total cholesterol content between the microclimatic temperature of 12° to 14°C or 24° to 26°C. However, he observed a significant hypercholesterolemia between birds of five to eight weeks of age. Donkoh (1989) noted hypercholesterolemia in birds exposed to heat and cold stress.

Cetin and Tuncel (1995) concluded that stress symptoms including increased concentration of plasma total cholesterol could occur in chicks of six and eight weeks of age, when stocking density increased to 22 chicks/m^2 or more. Reddy (2003) did not observed any significant change in the plasma total cholesterol value in 42 and 56 days old broilers maintained at a stocking density of 22 birds/ m^2 for a period of 14 days.

It was seen that corticosterone injection for two weeks increased plasma total cholesterol in chicken (Nagra and Meyer, 1963). Latour *et al.* (1996) and Puvadolpirod and Thaxton (2000b) obtained hypercholesterolemia on continuous infusion of ACTH at the rate of 16 IU per kilogram body weight per day for seven days.

Freeman *et al.* (1984) found that transporting broilers upto 224km produced a transient hypocholesterolemia followed by hypercholesterolemia in both summer and winter. Zulkifli *et al.* (2000c) reported hypocholesterolemia in feed restricted (40, 60 and 80%) and heat-treated (38±1^{0}C) broiler chicken, when compared to those fed *ad libitum.*

2.2.2.3 Blood Glucose Concentration

Freeman (1978) noted hyperglycaemia in newly hatched chicks exposed to heat stress (40°C for 1hour) while hypoglycaemia was observed by Edens (1978) when young birds were exposed to 42°C for 90 min. Donkoh (1989) reported hypoglycaemia in birds under environmental stress. However, Deyhim *et al.* (1995) could not observe any change in the blood glucose level in birds under heat stress.

In broilers, increasing the housing density from 10 to 14 birds/m^2 to 22 birds/m^{2}produced hyperglycaemia (Cetin and Tuncel, 1995). However, Reddy (2003) did not find any significant change in blood glucose level in 42 and 56 days old broilers at a stocking density of 22 birds/m^2.

In turkeys, excitement resulted in 44 per cent increase in blood glucose level whereas intramuscular injection of corticotropin at the rate of 20 IU/kg body weight caused 58 per cent increase in blood glucose level (Jaksch and Stix, 1982). Hyperglycaemia was also observed in birds following continuous infusion of ACTH at the rate of 8 IU/kg body weight/day (Latour *et al.*, 1996 and Puvadolpirod and Thaxton, 2000a). Later, Puvadolpirod and Thaxton (2000c), in their study in broilers concluded that continuous infusion of ACTH (16 IU/kg body weight/day for 7 days) elevated the plasma glucose level by 12h.

Stress increased circulating glucocorticoids, which in turn elevated the blood glucose concentration (Siegel, 1961; Siegel, 1971 and Chatterjee, 1994). This hyperglycaemic response to stress was seen only when the stressor was relatively strong (Davison, 1975). It was observed that transportation for 16 to 18 km (Halliday *et al.*, 1977) and vigorous exercise (Saleh and Jaksch, 1977) could result in hyperglycaemia. Freeman *et al.* (1984) revealed that transporting broilers for two to four hours and 224 km produced an initial hypoglycaemia followed by hyperglycaemia.

2.2.3 Plasma Hormonal Profile

2.2.3.1 Corticosteroids

2.2.3.1.1 Corticosterone

Edens and Siegel (1976) reported a rapidly decreasing blood corticosteroid profile in chickens exposed to high environmental temperature (45^0C for 2h). However, Edens (1978) observed an increased plasma corticosteroid concentration in chicks after exposure to 42°C for 90min, which then declined rapidly. Siegel (1980) suggested that corticosteroid concentration in the blood could be used as a measure of environmental stress and physiological activity in chicken. He attributed this to acute adrenocortical insufficiency developed in heat-stressed chicken. Gould and Siegel (1985) recorded an increased serum corticosteroid concentration in broilers subjected to heat stress.

Siegel and Gould (1982) opined that prolonged and repeated exposure to heat would reduce corticosteroid response to heat stress. Hill (1983) had the opinion that long-term response to stress involved the release of ACTH from adenohypophysis, which stimulated adrenal cortex to produce corticosterone. On the other hand, Gross and Siegel (1983) opined that H/L ratio was a better measure of long-term changes in the environment and changes in blood corticosterone concentration was a better measure of short-term stress. Correlation between H/L ratio and plasma corticosterone level were the best in stable, relatively low stress environment.

Kozma and Kemeny (1972) and Wise and Frye (1973) opined that the concentration of corticosterone in the plasma increased during the later stages of incubation and reached a maximum in the early post-hatching period. Thereafter, by the time the bird matured, the value was lowered by a factor of five. El-Halawani *et al.* (1973) noticed a diurnal rhythm in the plasma corticosterone concentration. They suggested that corticosterone concentration, which increased shortly after exposure to stress, returned to its initial concentration after two to three weeks depending on the intensity of stressor. Later it was supported by the experiments of Buckland *et al.* (1974) and Edens and Siegel (1975), where they observed an increased circulating level of corticosterone concentration shortly after exposure to a stressor.

Beuving and Vonder (1978) observed an increased plasma corticosteroid level within minutes after injection of corticotropin into young or old laying hens or cockerels. This increase was more rapid in younger than in older birds. Later Broom and Knowles (1989) reported an increased plasma corticosterone concentration in laying hens on handling and hand immobilization stress. Littin and Cockrem (2001) observed an increased plasma corticosterone concentration (5.47ng/ml) 15 min after repeated handling stress of laying hens, which declined to basal level (2.58ng/ml) 40 min after first handling.

Freeman *et al.* (1984) reported that transporting broilers for two to four hours and about 220km resulted in higher plasma corticosterone concentration (4.5ng/ml against 1ng/ml in control birds) and the increase was more in summer than in winter. Increased plasma corticosterone concentration was also observed in layers subjected to social stress (Koelkebeck and Cain, 1984). They also reported an increased corticosterone level in birds reared in floor pens at a floor space allowance of $0.094m^2$/bird, when compared to that at a floor space allowance of $0.373m^2$/bird. By this study, it was concluded that the number of birds kept per cage would affect the physiological responses of birds to social stress.

Lagadic *et al.* (1990) by their experiment with caged layers in groups proved that the time taken to remove the blood sample did not influence the circulating corticosterone concentration. The study also revealed that, it was possible to take blood samples from birds kept in groups without affecting the plasma corticosterone concentration in other birds from that group or in birds from other groups in nearby cages.

Latour *et al.* (1996) reported that continuous infusion of ACTH (8 IU/kg body weight/day) in chicken resulted in increased plasma corticosterone level. This was again confirmed by Puvadolpirod and Thaxton (2000a), when they observed an increased plasma corticosterone concentration in broilers by two hours in response to continuous infusion of ACTH (16 IU/kg body weight/day for 7 days). On the other hand, Guemene *et al.* (2001) could not observe any significant increase in plasma corticosterone concentration in male Mule ducks subjected to force-feeding.

2.2.3.1.2 Plasma Cortisol

Gill and Sharma (1992) opined that cortisol level in broiler chicken did not differ significantly between stocking densities (0.75 and 1sq.ft/bird). Reddy (2003) reported a significant increase in plasma cortisol level in broilers subjected to overcrowding stress (492 cm^2/bird) when compared to the control birds (734 cm^2/bird). He also observed a higher cortisol level (5.419 ng/ml) in birds at sixth week of age than those at eighth week (5.00 ng/ml), and he attributed it to increased resistance to stress with age. According to him, in broilers stress due to feed restriction increased the plasma cortisol concentration.

2.2.3.2 Tri-iodothyronine (T_3) and Thyroxine (T_4)

Heninger *et al.* (1960), Rogler and Parker (1978) and Bowen *et al.* (1984) reported a reduced concentration of T_3 and T_4 in heat-stressed chicken, while an increased level was noted by May *et al.* (1986) and Iqbal *et al.* (1990) due to heat stress in birds.

Kuhn and Nouwen (1978) noted an increased serum T_3 and T_4 concentration in 40 days old chicks, when the ambient temperature was reduced from 26.5° to 12.5°C. May (1978), Cogburn and Harrison (1980), Klandorf *et al.* (1981), Freeman and Cogburn (1983) and Yahav (2000) reported that plasma T_3 level decreased in response to increase in ambient temperature. Contrary to this, Rudas and Pether (1984) and Williamson *et al.* (1985) could not observe any change in plasma T_3 concentration in birds under increased ambient temperature. Sinurat *et al.* (1987) studied the effect of high ambient temperature (35°C) on plasma concentration of T_3 and T_4 in 21 days old broiler chicken and concluded that exposure of chicks to 35°C for five hours per day increased T_4 and reduced T_3 level of plasma.

Bobek *et al.* (1980) observed a constant reduction in plasma T_4 concentration in Japanese quail subjected to warm ambient temperature, when compared to those at thermoneutral environment. An increase in plasma T_3 concentration was evident five hours and 48 hours after exposure to warm temperature. Davison *et al.* (1985) opined that corticosterone produced during stress could affect circulating level of thyroid hormone too.

May (1982) found that broilers fed T_3 (0.5 ppm in feed) and T_4 (0.6 ppm in feed) had shorter heat stress survival times than non-treated controls. Bowen *et al.* (1984) suggested that the ability of young chicken to withstand heat stress is decreased in hyperthyroidism and increased in hypothyroidism.

2.2.3.3 Other Hormones

In mammals both physiological and psychological stressors increased plasma glucagon concentration (Bloom *et al.*, 1972; 1973). Later Freeman (1976) reported a similar response in glucagon concentration in domestic fowl.

Freeman (1980) opined that glucagon is a stress hormone in domestic birds and it was responsible for activating hypothalamo-hypophyseal adrenocortical axis.

2.2.4 Liver Function

2.2.4.1 Plasma Enzymes

Siegel *et al.* (1974) reported that serum Alkaline phosphatase (ALP) level of birds reared at 35°C was significantly higher than that reared at 32°C. Polonis (1982) in his experiment on the effect of environmental temperature on some blood biochemical indices of chicks observed a highly significant increase in ALP activity between a micro-climatic temperature of 12° to 14°C and 18° to 20°C. He also observed a significant increase in Aspartate amino transferase (AST) activity from 288 KU at 12° to 14°C to 366.8 KU at 24° to 26°C. In the same study the lowest Alanine amino transferase (ALT) value of 29.1 KU was observed at 12° to 14°C and the highest value of 60.1 KU at 24° to 26°C, without any significant difference in enzyme activity between males and females. Fenghua *et al.* (1997) reported a significantly lower value of ALT at 34.5°C compared to that at 26°C.

Hocking *et al.* (1994) observed an increased creatine kinase activity in 52 days old broilers under induced thermal stress (32°C and 40% relative humidity for three hours exposure) when fed *ad libitum* or restricted. Chunyan *et al.* (1998) studied the effect of environmental temperature on selected enzyme activity in chicken. They observed a reduction in the serum glutamate pyruvic transaminase (SGPT) and serum ALP concentration after exposure of fowls to high ambient temperature, whereas they could not found any significant effect of high ambient temperature on lactate dehydrogenase (LDH) activity.

2.2.5 Immunity

Stress caused involution of lymphoid organs such as thymus, spleen, and bursa of Fabricius, which reduced the number of circulating lymphocytes and increased number of heterophil granulocytes (Garren and Shaffner, 1956;

Glick, 1967; Siegel, 1980; Siegel, 1983). Thaxton and Siegel (1973) reported that the antibody production was impaired by stressor, which did not even evoke a rise in plasma corticosterone concentration. Later it was confirmed by Mashaly *et al.* (2004), who observed a reduced antibody production in laying hens exposed to constant heat and humidity (35^0C and 50% relative humidity).

Thaxton *et al.* (1968) were the first to demonstrate that high environmental temperature (44.4°C to 47.8°C) affected the development of specific immune responses in young chickens. These effects included suppression of circulating leukocytes (Nathan *et al.*, 1976; Heller *et al.*, 1979) and an increase in H/L ratio (Saleh and Jaksch, 1977), which is an indicator of stress (Gross and Siegel, 1983). Zabudskii and Skuttar (1993) observed a fall in migratory activity of leukocytes in cold-stressed birds. They reported that migratory activity of leukocytes could be considered as an indicator of stress in birds.

Heller *et al.* (1979) observed significantly increased antibody titre against sheep red blood cells (RBC) following heat exposure. Reduced antibody production to sheep RBC was reported (Gross *et al.*, 1980; Zulkifli *et al.*, 2000a) in young chicken (31 days of age) subjected to heat stress (38 ± 1^0C for 2h/day for 7 days)). However, Donker *et al.* (1990) found that heat exposure (intermittent exposure to 40^0C and 22^0C at 30 min. intervals) did not reduce antibody production to sheep RBC.

Regnier *et al.* (1980) suggested that heat-induced immunosuppression might depend on the breed of the bird. Kelly (1983) opined that effects of heat on immune responses might depend on the length and intensity of heat exposure. Pardue and Thaxton (1984) reported that it was the T-cell population that was mainly affected by the immunological response. As per Blalock and Smith (1985) and Campos *et al.* (1991), the stress responses could be modified through the endocrine functions of the immune system.

The catecholamines and corticosterone produced in response to heat stress in birds would initiate peroxidation in cell membranes including membranes of B and T-lymphocytes (Freeman and Crapo, 1982). The elevated glucocorticoids in stressed animals were responsible for suppressing the production of interleukin-2 by lymphocytes, which was an essential proliferative factor that impaired the lymphocyte blastogenic response (Bleecha *et al.*, 1984). Reddy (2003) observed a reduced lymphoblastogenic response in birds at increased housing density of 22 birds/m^2 when compared to 14 birds/ m^2

Swenson and Reece (1996) suggested that one of the physiological responses of exposure to stress was the release of glucocorticoids causing dissolution of lymphocytes in lymphoid tissue leading to lymphopenia.

2.2.6 Production Parameters

Tomhave and Seegar (1945) observed a lowered body weight in birds subjected to stress by floor space reduction. However, Siegel and Coles (1958) did not observe any significant difference in body weight and feed efficiency of broilers reared at various floor space levels ranging from 0.5 to 1.25 sq.ft/bird. Mandlekar and Thatte (1986) could not observe any appreciable difference in body weight of broilers at a floor space of 0.48, 0.53, 0.58 or 0.61 sq.ft/bird. However, Quinones *et al.* (1987) reported reduced growth rate in caged broilers, when stocking density was increased. But the increased stocking density did not affect the survival percentage. Shanawany (1988) noted a linear decrease in feed intake with densities over 20 birds/m^2 with significant improvement in feed efficiency in the birds housed at high stocking densities.

Bolton *et al.* (1972) reported that decrease in space allowance from 0.093 to 0.047m^2/bird was accompanied by a reduced final live weight. Bhargava *et al.* (1975) reported that caged broilers when provided with the floor space of 0.7 sq.ft/bird, weighed significantly less than those reared at densities of 0.5 sq.ft /bird. On the other hand, Sulane and Ledaiyc (1976) observed no marked difference in body weight of broilers in cage densities of 0.5 to 0.65 sq. ft/bird. Weaver *et al.* (1982) reported a significant reduction in body weight at fourth week of age at a stocking density of 0.07 m^2/bird when compared with the body weight of birds at 0.11 m^2/bird stocking density. Biligili and Hess (1995) suggested that body weight would improve significantly, when birds were given more space. This was later confirmed by

Fedder *et al.* (2002) and Dawkins *et al.* (2004) when they obtained an improved live weight and carcass weight on reduction of stocking density.

Alribdawi and Singh (1989) reported that upto four weeks of age, stocking density had no significant effect on the body weight of broilers, when reared in groups of 10, 15 or 20 birds/m^2. Kuan *et al.* (1990) observed 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 the feed efficiency without affecting the growth rate. After the sixth week, birds at lowest stocking densities had the highest average daily gain (39.20g) and daily feed consumption (127.00 g) and poorest feed efficiency (3.30g feed/g gain). Birds at the highest stocking density had the lowest average daily gain (32.20 g) and feed consumption (90.60 g). They concluded that stress associated with overcrowding reduced the performance in broilers. Sorenson *et al.* (2000) noted that high stocking density was associated with reduced live weight, poor walking ability and more foot and hock burns.

Cravener *et al.* (1992) reported that population density had no effect on feed conversion at sixth or seventh week of age. However, they observed a higher body weight for birds housed at a stocking density of 0.07, 0.09 and 0.11m^2/bird, when compared to those at 0.05m^2/bird, indicating stress at higher stocking densities. Gill and Sharma (1992) opined that the stocking density (0.75sq.ft and 1sq.ft/bird) did not significantly affect the body weight gain of broilers at sixth week of age in both deep litter and on wooden slat floors. At eighth week of age, birds at floor space of 1sq.ft. /bird was better than 0.75sq. ft./bird and the birds on slat floors were significantly heavier and consumed more feed at both the stocking densities than the birds reared on the deep litter system. They could not observe any significant difference in the feed efficiency of birds either between floor systems or stocking densities.

Deaton *et al.* (1978), Reece and Lott (1983), Sinurat and Balnave (1985) and Suk and Washburn (1995) revealed that an increased environmental temperature reduced feed consumption and growth of meat type poultry.

Donkoh (1989) found that exposure of birds to temperatures of 20, 25, 30 and 35°C resulted in a significant lowering of the growth rate. However, the mortality was not affected by environmental temperature ranging from 20°C to 35°C. Jaffar and Blaha (1996) and Sokolowicz *et al.* (1996) observed a reduced live weight and increased mortality percentage in heat-stressed birds. Al-Batshan and Hussein (1999) also reported that the cyclic high temperature (26 to 34°C for 6h, 34±1°C for 12h and 34 to 26°C for 6h) reduced the body weight and feed efficiency.

Exposure of 21 days old broiler chicks to high ambient temperature (35°C) for five hours per day for one month significantly reduced the body weight. The reduction in body weight was due to reduced feed intake at high ambient temperature (Sinurat *et al.*, 1987). Yahav and Hurwitz (1996) demonstrated that as the period of thermal conditioning was extended, the compensatory growth response deteriorated.

Study by Thankson *et al.* (2001) recorded that heat stress (24 to 34°C and RH of 15%) and ACTH infusion (8 IU ACTH/kg body weight for seven days) produced the same effect on body weight, and both reduced the body weight in broilers. As per Yahav and Mc Murtry (2001), short-term exposure of chicks to mild heat stress during the first week post-hatch (36±1°C and 70-80% RH for 24h at five days of age) resulted in the retardation of growth followed by an immediate compensatory growth phase. Mashaly *et al.* (2004) studied the adverse effects of high temperature and R.H on 31 week old commercial laying hens. They reported that heat stress (constant heat and humidity of 35^{0}C and 50% R.H) for five weeks reduced the body weight and feed consumption in laying hens.

Spinu *et al.* (1992) opined that when broilers were transferred from large runs to be caged in pairs, the body weight reduced during the first 10 days but not in the following 16 days. Puvadolpirod and Thaxton (2000c) concluded that experimental induction of stress by continuous administration of ACTH (8 IU/day i.v for seven consecutive days) resulted in decreased body weight, feed intake and reduced the relative weight of bursa, thymus and spleen. They also observed that body weight did not return to the normal value one week after cessation of ACTH administration.

2.3 GROWTH PROMOTERS IN POULTRY PRODUCTION

2.3.1 Antibiotics

Soon after the introduction of antibiotics into human chemotherapy in 1940s, they were also introduced into veterinary practice (Moore *et al.*, 1946). In the poultry industry, three to five per cent increase in the growth rate and feed conversion efficiency was achieved by the use of antibiotics in feed at prophylactic levels (Thomke and Elwinger, 1998).

By 1949, antibiotics had been approved for growth promotion in experimental, and many different groups of antimicrobials have subsequently been approved for on-farm use as growth promoters in many European countries (Inborr, 2000). However, it is suggested that antibacterials used for animal feeding became risky for animal (Thorns, 2000) and human (Sahin *et al.*, 2002) health and the conclusions of the scientific committee of the European council had led to a ban on feeding antibiotics (Witte, 2000). But, in India many antibiotics such as tetracycline, zinc bacitracin, virginiamycin etc. are widely used as antistress agents and growth promoters in livestock and poultry.

The antimicrobial activity of virginiamycin was first reported by Desomer and Van Dijek (1955) and was subsequently shown several years later to have additional potential as a growth promoter in chicken when used at sub-therapeutical levels (Eyssen *et al.*, 1962 and Eyssen and Somer, 1963). Combs and Bossard (1963) recorded a better growth rate in broilers fed with virginiamycin when compared to other antibiotics. But Freeman (1976) opined that antibiotics did not alleviate stress. Proudfoot *et al.* (1990) suggested that supplementation of virginiamycin at the rate of 11mg/kg in feed or drinking water to broilers did not produce antibiotic residues in the meat.

2.3.2 Probiotics

Increased public concern over the development and spread of antibiotic resistance in bacteria and the possible presence of antibiotic residues in poultry products had led to the search for alternatives to replace antibiotics in the chicken diet (Gong *et al.*, 2002). This resulted in the introduction of probiotics in the poultry feed.

Probiotics are health-promoting bacteria inhabiting the gastrointestinal tract of humans and animals. Exactly how these supplemental dietary microbial products function in the digestive system is not known. The positive effects of probiotics on animals resulted either from a direct nutritional effect of the probiotics or a health effect with probiotics acting as bio-regulators of the intestinal microflora and reinforcing the host's natural defense (Karaoglu and Durdag, 2005).

Tortuero (1973) first found that *Lactobacillus* cultures improved broiler growth. Later, Watkins and Kratzer (1984), Jernigan *et al.* (1985) and Dawson (1993) conducted experiments to determine the effects of probiotics on performance of poultry and suggested that supplementing broilers with microbial cultures provided beneficial bacteria that aided in nutrient absorption and enhanced the microbial balance in the avian digestive tract. Reports of Erdogan (1999) and Midilli and Tuncer (2001) showed that additional benefits could be gained by supplementing broiler diets with probiotics as feed additives. Kutlu and Gorgulu (2001) suggested that probiotics could be used to get rid of stress-induced abnormalities in the gastrointestinal tract.

2.3.3 L-Ascorbic Acid (Vitamin C)

The birds can synthesize vitamin C in their body. Hence there is no recommended requirement of vitamin C for avian species. However, the synthesis becomes inadequate especially under stressful conditions. Environmental and pathological stressors are known to alter vitamin C synthesis and utilization in birds (Pardue and Thaxton, 1986; Kutlu and Forbes, 1993). Administration of ascorbic acid had a protective effect against stress (Satterlee *et al.*, 1989; Gross, 1992). It could be used to attenuate the negative effects of stress, hence reducing mortality (Njoku, 1986) and improving the growth rate (Sahin and Kucuk, 2001). Ascorbic acid is probably the most effective and least toxic antioxidant identified in mammalian system (Rakeshkumar *et al.*, 2001). Vitamin C is not an essential nutrient for chicks maintained under optimal conditions but it may become an essential vitamin under environmentally, pathologically or nutritionally stressful conditions.

2.4 EFFECT OF GROWTH PROMOTERS ON PHYSIOLOGICAL PARAMETERS

2.4.1 Antibiotics

2.4.1.1 Haematological and Blood Biochemical Parameters

Dash *et al.* (1992) observed a non-significant decrease in the values of Hb content (9.89g% against 10.10g% of control birds) and VPRC (27.70% against 29.20% of control group) in broilers fed with Stafac-20 (virginiamycin) at the rate of 10g per 100 kg feed. Odunsi and Onifade (1998) as well as Odunsi *et al.* (1999) stated that in birds supplemented with virginiamycin, the haematological values varied within the normal range. However, Burke and Culha (2003) and Ali *et al.* (2005) concluded that erythrocytes were microcytic in birds treated with antibiotic. They have also opined that antibiotics had a tendency to produce microcytosis and anaemia.

Burke and Culha (2003) reported that leukopenia occured in birds fed with antibiotics. Ali *et al.* (2005) observed reduced values of volume of packed red blood cells (VPRC), red blood cell (RBC), concentration of haemoglobin (Hb), WBC, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in antibiotic treated birds. However, the values in both groups were within the normal range. Their investigations revealed that antibiotic treatment caused a fall in haematological parameters in baby chicks (1 to 5 days), whereas in older birds (22 to 27 days) the values were analogous with normal range. The authors attributed this to incomplete development of haematopoietic system.

Buyse *et al.* (1991) concluded that clenbuterol supplementation (42 mg/100 kg feed) for six weeks resulted in reduced T_4 concentration (24.3ng/ml) in female broiler chicks. The plasma T_4 level increased with age, which was also pronounced in female birds. Supplementation of clenbuterol upto four weeks increased the plasma T_3 level in females whereas it reduced the T_3 concentration in males. The plasma level lowered significantly in male broilers on clenbuterol treatment for two weeks, whereas the concentration of triglycerides increased in females fed with clenbuterol for four to six weeks and males supplemented with clenbuterol for six week period.

2.4.1.2 Production Parameters

Waibel *et al.* (1954) and Sherman *et al.* (1959) reported that continuous use of antibiotics for growth promotion of chicks resulted in a decrease in their growth performance. However, Yates and Schaible (1961) concluded that virginiamycin was quite effective in promoting the growth of chicks and poults. Combs and Bossard (1963) observed a better growth rate in broilers fed with virginiamycin when compared to other antibiotics. Contrary to this, Freeman *et al.* (1975) failed to observe any beneficial effects for antibiotics to alleviate metabolic response to various stressors. Later, Freeman (1976) opined that antibiotics did not alleviate stress.

March *et al.* (1978) observed that virginiamycin (22ppm) in feed stimulated the growth of chicks but was not active against *Enterobacteriaceae*. Therefore, virginiamycin was not involved in resistance transfer among these organisms, which was an important advantage from the standpoint of public health. It is suggested that incorporation of virginiamycin at the rate of 10 to 22 mg/kg in feed improved the growth rate and feed conversion ratio in broilers (Miles *et al.*, 1985). However, Proudfoot *et al.* (1990) did not observe any significant increase in the growth rate and feed efficiency in broilers supplemented with virginiamycin at the rate of 11 mg/kg of diet. They noted that, virginiamycin supplementation at this rate would not produce antibiotic residue in the meat.

Nelson *et al.* (1963) suggested that growth stimulatory effect of antibiotics resulted from increase in efficiency of absorption of calorigenic nutrients. March *et al.* (1978) observed a significantly increased weight gain in broilers supplemented with virginiamycin at the rate of 22 ppm in the feed. A similar response was again reported by Singh *et al.* (2000) in their study in broilers fed with virginiamycin at the rate of 500g/MT feed upto 42 days of age. Buresch *et al.* (1985) recorded an increased body weight, feed efficiency and dietary energy utilization in turkey poults supplemented with virginiamycin at the rate of 22 mg/kg feed, when fed *ad libitum* as well as feed restricted by 25 per cent. Harms *et al.* (1986) observed a significant increase in body weight in seven week old Cobb Feathered sexed broiler chicks fed with two rations varying in metabolizable energy (ME) and supplemented with virginiamycin at the rate of 16.5 mg/kg feed. The increase in weight gain was more with low energy ration. Sivakumar *et al.* (2001) in their study in broilers observed that supplementing virginiamycin to diets with suboptimal protein or energy resulted in improved broiler performance.

Raynaud (1997) reported that antibiotic supplementation in animals and birds increased body metabolism, enhanced feed intake and improved the weight gain. Dash *et al.* (1992) and Singh *et al.* (2000) revealed that antibiotic supplementation improved the growth rate in broilers. On calculation of economy of production per bird, a better gain was observed in virginiamycin fed group of birds when compared to other antibiotics fed groups (Singh *et al.*, 2000). Shojadoost *et al.* (2001) observed a better body weight, weight gain and feed efficiency in antibiotic fed broilers when compared to control birds. They could not observe significant variation in weight gain between different antibiotics fed groups.

2.4.2 Probiotics

2.4.2.1 Haematological and Blood Biochemical Parameters

Pollmann *et al.* (1980) noticed elevated levels of total serum proteins, globulin and WBC on oral inoculation of *Lactobacillus acidophilus* in germ free pigs. Mc Dowell (1989) opined that probiotic compete with the host for folic acid, which might cause anaemia in probiotic supplemented animals and birds. Mohan *et al.* (1996) confirmed this when they observed anaemia in broilers supplemented with probiotic. However, they could not observe any significant change in VPRC value on dietary supplementation of probiotic.

Gohain and Sapcota (1998) reported that probiotic supplementation at the rate of 50g/100kg diet did not produce any significant change in serum protein concentration in broilers. The serum cholesterol concentration reduced numerically, but not significantly. Biswal *et al.* (2000) and Sabiha (2001) found that dietary supplementation of probiotic did not influence the serum protein levels in broilers at eight weeks of age.

Rao *et al.* (1981) and Grunewald (1982) reported that probiotic supplementation reduced serum cholesterol in rats. However, Samanta and Biswas (1997) did not observe any change in the serum protein level in caged broilers supplemented with probiotic (*Lactobacillus acidophilus, Lactobacillus bulgaricus* or both). Abdulrahim *et al.* (1996) opined that, *Lactobacillus acidophilus* reduced the serum cholesterol concentration in the blood by de-conjugating bile salts in the intestine thereby preventing them from acting as precursors in cholesterol synthesis.

Mohan *et al.* (1996) evaluated the effect of probiotic at 75, 100 and 125mg/kg broiler diet upto eight weeks of age and noted that serum cholesterol was significantly reduced in probiotic fed groups. Birds, which received 100mg probiotic/kg diets, had the lowest cholesterol level (84.1mg/dl). As per Jin *et al.* (1998), supplementation of *Lactobacillus* cultures at 0.1 per cent level significantly reduced the serum cholesterol levels at 40 days of age. They concluded that the decrease might be due to cholesterol assimilation by *Lactobacillus* cells. Endo *et al.* (1999) observed that the serum cholesterol level was significantly reduced in cocks fed with a cholesterol-enriched diet and supplemented with probiotic (mixture of *Bacillus, Lactobacillus streptococcus, Clostridium, Saccharomyces* and *Candida).*

2.4.2.2 Production Parameters

Mohan *et al.* (1996) opined that probiotic feeding in broilers significantly increased the body weight at fourth and sixth week of age but not at eighth week of age. However, they could not observe any significant difference in the dressing per cent and weight of internal organs in probiotic supplemented group. Pradhan *et al.* (1998), Richter *et al.* (2000) and Banday and Risam (2001) observed a better performance with probiotic feeding whereas Ladukar *et al.* (2002) and Karaoglu and Durdag (2005) could not observe a better weight gain on probiotic feeding. Kannan and Viswanathan (2000) observed a significant increase in body weight in probiotic-supplemented broilers when compared to the control group. Chithra *et al.* (2004) obtained a highly significant increase in body weight and feed conversion in broilers on dietary supplementation of probiotics at the rate of 0.30g/60 chicks.

Takalikar *et al.* (1992), Baidya *et al.* (1994) and Mandal *et al.* (1994) reported that feeding probiotics did not produce any effect on carcass yield. Samanta and Biswas (1997) inferred that *Lactobacillus bulgaricus, Lactobacillus* *acidophilus* or a combination of both at $10x10^{12}$ number of organisms/l of water in caged broilers upto six months had no effect on the dressed weight and eviscerated percentage as well as weight of giblet and spleen. Choudhury *et al.* (1998) observed no significant difference in dressing percentage, giblet yield and weight of liver, heart and gizzard on supplementing broilers with probiotic growth promoters at 0.05 per cent level.

Buche *et al.* (1992) revealed that inclusion of 0.02 per cent probiotic to broiler diet numerically lowered the cost of feed per kilogram live weight compared to that of the control and the higher level of 0.04 per cent supplementation. Takalikar *et al.* (1992) and Prasad and Sen (1993) reported that the cost of feed per kilogram of weight gain was higher in probiotic supplemented group (0.021 and 0.5g/kg feed).

Chiang and Hsieh (1995) noted that supplementing probiotic at the rate of 0.25 and 0.5g/kg diet upto six weeks produced no significant difference in the abdominal fat content. Singh and Panda (1995) observed that the probiotic fed animals had lower fat content and higher protein content, when compared to non-supplemented birds.

According to Karaoglu and Durdag (2005), probiotic supplementation did not improve the body weight gain. It reduced subcutaneous and intramuscular fat deposition. However, they could not observe any change in the abdominal fat pad thickness or fat pad weight, which was an indicator of carcass fatness in birds.

Singh and Sharma (1999) concluded that *Lactobacillus sporogenous* at the level of 0.02 per cent in a feed with 2600kcal metabolizable energy (ME) per kilogram provided optimum live weight and feed efficiency in summer. Mahajan *et al.* (2000) suggested that supplementing the diet with probiotic under high environmental temperature improved feed consumption and feed efficiency and reduced mortality.

In the study by Zulkifli *et al.* (2000a), it was noticed that broiler chicks that received *lactobacillus* cultures had better feed efficiency followed by those received oxytetracyclin and control birds at day one to twenty one. But after three weeks of heat exposure, the birds received *lactobacillus* culture diet had greater body weight and weight gain but lower feed efficiency, when compared to oxytetracyclin fed group and control group.

As per the reports of Mudalgi *et al.* (1993), feeding *Lactobacillus acidophilus* or *Lactobacillus bulgaricus* (10×10^8cells/l of drinking water) to broiler chicks upto six weeks of age did not produce any significant change in the body weight gain, feed intake or feed efficiency. Findings of Ergun *et al.* (2001) suggested that in broilers supplementation of probiotic with or without antibiotic had no effect on live weight gain, feed efficiency, carcass yield and edible visceral organ weight. Banday and Risam (2001) noted that supplementing probiotic at the rate of 75g per 100kg feed improved growth rate, feed efficiency, carcass yield and profitability. However, Ladukar *et al.* (2002) could not observe any significant difference in feed intake, live weight gain and feed conversion ratio and nitrogen retention on probiotic supplementation.

According to Baidya *et al.* (1994) and Mandal *et al.* (1994), supplementing probiotic in broiler ration did not improve carcass yield. Gohain and Sapcota (1998) reported that probiotic feeding at the rate of 50g/100kg diet significantly improved body weight but it had no effect on feed efficiency.

2.4.3 Ascorbic Acid (Vitamin C)

2.4.3.1 Haematological and Blood Biochemical Parameters

Gross (1988; 1992), Satterlee *et al.* (1989) and Pardue and Williams (1990) opined that vitamin C supplementation reduced the H/L ratio in poultry in conditions such as heat stress, disease or pre-slaughter stress. Mc Kee and Harrison (1995) in their study on the effect of supplementing ascorbic acid on the performance of broiler chicken exposed to multiple stressors concluded that ascorbic acid supplementation lowered the plasma corticosterone and H/L ratio. Zulkifli *et al.* (2000b) could not observe any significant difference in H/L ratio in birds supplemented with ascorbic acid (1200ppm) and control group at zero hour, whereas 20h after either upright or inverted handling treatment, ascorbic acid supplemented birds had lower H/L ratio than control. Ascorbic acid supplemented birds also showed reduced fearfulness.

Zulkifli *et al.* (2001) studied the effect of road transportation on stress in broiler chicken concluded that H/L ratio was significantly lower in broilers supplemented with 1200ppm L-ascorbic acid than the control birds after transportation for 40min. in an open truck.

Ascorbic acid promoted mineral mobilisation from bones (Thornton, 1970) and increased plasma Ca^{++} concentration (Sifri *et al.*, 1977). Sahin *et al.* (2002) suggested that the effects of ascorbic acid on retention of nitrogen and minerals could be attributed to the protection of pancreas from oxidative stress. Sheila and Cheryl (1978) in their study in day-old White Leghorn chicks noticed that ascorbic acid in the diet did not significantly alter corticosteroid response to ACTH stimulation in immature birds. The immature birds responded to ACTH injection with higher corticosterone levels than mature birds of either sex. Mature birds fed with 3000ppm ascorbic acid had a lower corticosterone level than others. Ascorbic acid might function as a regulator of steroid synthesis in adrenals as high adrenal ascorbic acid had been found to inhibit steroidogenesis (Samuel *et al.*, 1985).

Pardue *et al.* (1985) reported that immunosuppression due to high environmental temperature (22±0.80^{0}C) had been reduced by ascorbic acid supplementation at the rate of 1g/kg feed. It is suggested that ascorbic acid supplementation might alter endogenous synthesis and secretion of adrenal steroids thereby limiting the lymphotoxic effects associated with glucocorticoids or served to protect immunological tissue from steroid insult.

Kutlu and Forbes (1993) opined that ascorbic acid reduced the synthesis of corticosteroid hormone in birds and Sahin *et al.* (2002) observed a low concentration of ACTH in quails reared at 32°C and fed with a diet with vitamin C. Satterlee *et al.* (1993) in a study in 18 days-old Japanese quails subjected to stress for 12 to 21h with a reduction in floor space observed an increased corticosterone production. But supplementation of vitamin C at the rate of 1200ppm in drinking water failed to affect the adreno-cortical response to cooping combined with overcrowding.

Marcusen and Heninger (1976) reported that injection of vitamin C in rats caused a reduced thyroid function. Ladmacki *et al.* (1997) observed a significant reduction in the plasma T_4 levels at five and six weeks of age by dietary vitamin C supplementation (500ppm). However, they noticed a significantly higher T_4 concentration in the vitamin C supplemented birds at four weeks of age. They also noted that supplementing vitamin C at the rate of 500ppm reduced plasma thyroid hormone levels without affecting the value of haematocrit and blood gas levels in day-old broilers fed with T_3 supplement.

Mc Corkle and Glick (1980) suggested that ascorbic acid might modulate the B-lymphocytes but not T-lymphocytes. Pardue and Thaxton (1984) reported that supplemental ascorbic acid at the level of 1000ppm ameliorated the immunosuppression caused by cortisol in chicken. Franchini *et al.* (1994) found that by increasing the intake of vitamin C during early growth period antibody formation and per cent of deoxyribonucleic acid synthesizing cells were increased. Puthpongsiriporn *et al.* (2001) reported that combination of 65 IU alpha-tocopherol/kg and 1000ppm ascorbic acid in feed of hen had an additive improvement in *in vitro* lymphocyte proliferation in response to Con-A and LPS mitogen when compared with alpha-tocopherol or ascorbic acid supplementation alone.

Degkwitz (1987) noted that the serum cortisol concentration increased with a decline in vitamin C supplementation below 90mg per cent in guinea pigs. The portion of vitamin C excreted into the urine was progressively reduced in the birds supplemented with vitamin C beyond 90mg per cent. This indicated that high vitamin C supplementation induced an increased rate of its catabolism. According to Ginter and Jurcovicova (1987), vitamin C is necessary for the transformation of cholesterol to bile acids as it affected the rate limiting reaction of cholesterol catabolism.

Mc Kee *et al.* (1997) reported that ascorbic acid supplementation increased the plasma concentration of triglycerides in feed deprived birds when compared to control birds. They concluded that ascorbic acid supplementation influenced body energy stores that were used for energy purposes during periods of reduced energy intake. Elmoty *et al.* (1999) studied the effects of ascorbic acid administration during cold stress and found that ascorbic acid injection increased concentration of total proteins, glucose, T_3 and ascorbic acid in the blood, gonads, kidneys, spleen and thyroid gland. According to Gursu *et al.* (2004), vitamin C supplementation in heat-stressed broilers reduced the serum concentration of cholesterol, triglycerides and glucose and increased total proteins and albumin.

Avici *et al.* (2005) studied the effect of ascorbic acid on the performance and some blood parameters of Japanese quail reared under hot climate (average temperatures of 32.72°C and 34.80°C). They concluded that inclusion of 500mg per kilogram of ascorbic acid in the diet prevented the heat stress in quails upto four weeks of age. They also observed that ascorbic acid did not affect the blood concentration of sodium ions (Na^+), potassium ions (K^+) and the values of haematocrit and Hb concentration.

2.4.3.2 Production Parameters

Ahmad *et al.* (1967) and Herrick and Nockels (1969) reported low mortality rate in birds supplemented with ascorbic acid in peak summer. However, they could not observe any beneficial effect of ascorbic acid supplementation in unstressed birds. This lack of response was also indicated by the results of Sifri *et al.* (1977) and Niekerk *et al.* (1989). Kafri and Cherry (1984) opined that ascorbic acid supplementation was beneficial during heat stress (32°C) but it produced poor performance at 23°C. According to Kassim and Norziha (1996), vitamin C supplementation in broilers reduced the effect of heat stress and improved the weight gain and feed efficiency. Sayed and Shoeib (1996) noted that ascorbic acid added in drinking water (0.50g/l) or diet (200mg/kg diet) benefitted heat- stressed broilers and reduced mortality related to heat stress and improved body weight by 6.8 per cent.

Dorr and Balloum (1951) in their study on the effect of dietary ascorbic acid supplementation in turkey at the rate of zero, 30, 300, 1500 and 3000ppm in feed observed that body weight gain was significantly improved by ascorbic acid supplementation. However, non-supplemented group had better feed conversion efficiency than ascorbic acid treated group. Freeman *et al.* (1983) observed no improvement in weight gain or survivability of birds when ascorbic acid was provided in the diet at a concentration of 1000mg/kg feed. Similar results were reported by Brown and Southern (1985) on ascorbic acid supplementation in chicken at one per cent level.

Pardue *et al.* (1985) in their study on role of ascorbic acid in chicks exposed to high environmental temperature concluded that, four week old male chicks exposed to 43±0.10°C and 40±2.00 per cent R.H for 30min on each of three consecutive hours showed a reduced plasma K^+ concentration, reduced body weight and increased cloacal temperature. Ascorbic acid supplementation at the rate of 1000mg/kg diet ameliorated many of the stress related responses.

Pardue and Thaxton (1984) reported that supplemental ascorbic acid significantly ameliorated the immunosuppression associated with exogenous cortisol. Later Njoku (1986) observed an improved growth performance in broilers, when the diet was supplemented with 200mg ascorbic acid/kg feed. Heat induced growth inhibition; mortality and immune suppression were all reduced by ascorbic acid supplementation. These effects were attributed to either a reduction in glucocorticoid out put or protection of cells from the cytotoxic effect of adrenal steroids.

According to Kafri *et al.* (1984), Pardue and Thaxton (1986) and Sahin and Kucuk (2001) vitamin C could be used to attenuate the negative effects of environmental stress and to improve the growth rate in broilers. Study by Abd-Ellah (1995) revealed that addition of ascorbic acid at the level of 250mg and 500mg per kilogram diet markedly reduced mortality (8%) compared with the control (28%). Ascorbic acid supplementation was able to neutralize the negative effects caused by heat stress. It improved nutrient utilisation leading to an increased production. Lustsenko *et al.* (2002) reported that high intracellular concentration of vitamin C prevented oxidation induced mutation in human beings.

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 supplemental ascorbic acid had small influence on egg quality with a reduction in mortality rate. Sahota *et al.* (1994) studied the effect of ascorbic acid on silver black and White Leghorn chicks exposed to heat stress (39°C) and observed a better feed intake and feed conversion efficiency. Behl *et al.* (1995) concluded that, in layers ascorbic acid at the rate of 0.44mg and 0.8mg / kg feed improved the feed conversion efficiency in extreme hot weather when the environmental temperature was 42.5°C. As per Sahin and kucuk (2001) and Sahin *et al.* (2002), dietary supplementation of vitamin C reduced the negative effects of environmental stress in poultry.

Maus (1986) studied the effect of vitamin C incorporation at zero, 250 and 500mg/kg feed in broiler diets on stress at stocking densities of 18 and 23birds/m^2 and with single handling of birds. The highest stocking density resulted in reduced weight gain, but was nearly compensated for the highest dose of vitamin C. With lowest stocking densities, vitamin C produced higher weight gain. Mc Kee and Harrison (1995) in their experiment on the effects of supplemental ascorbic acid on the performance of broiler chicken exposed to multiple concurrent stressors (beak trimming, coccidiosis, and heat stress) found that ascorbic acid (150ppm) increased feed intake and improved the growth rate in stressed broilers. Jaffar and Blaha (1996) observed that mortality rate was lower in birds supplemented with ascorbic acid (20mg/bird/day and 40mg/bird/day in drinking water), when compared to the non-supplemented group. According to them, vitamin C along with electrolyte supplementation improved the body weight gain by 4.1 percentage.

According to Kutlu and Forbes (1993), reduced ascorbic acid concentration was associated with stress and ascorbic acid administration could protect against stress (Gross, 1992; Satterlee *et al.*, 1989) and had no effect on performance parameters such as growth rate, feed intake and feed efficiency (Ladmackhi, 1997). Mohammed (1997) stated that ascorbic acid supplementation at the level of zero, 150, 300 and 450mg/kg feed improved body weight in 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.

Elmoty *et al.* (1999) studied the effect of ascorbic acid administration during cold stress and found that, ascorbic acid injection reduced mortality and improved body weight and feed conversion efficiency in chicken. Memon *et al.* (2001) in their study on the effect of two commercial antistress drugs on the growth of artificially stress induced broilers obtained a better weight gain, dressing per cent and feed efficiency for vitaminic–33 (vitamin C). They concluded that antistress vitamin at the rate of 5g/40 l water *ad libitum* could be effectively used for better growth of broilers. Lohakare *et al.* (2004) reported that retention and availability of vitamin C in feed was higher than that in water and vitamin C supplementation in summer was beneficial for broilers. It was observed that supplementing 10ppm and 20ppm vitamin C in the feed and 5ppm and 10ppm vitamin in water from day-old to six weeks of age resulted in better feed intake and improved growth rate when compared to control birds. However, feed efficiency was less in the group supplemented with ascorbic acid. The dressing per cent and abdominal fat were also higher in vitamin C supplemented group when compared to their counterparts.

Materials and Methods

3. MATERIALS AND METHODS

A study was carried out in the Department of Physiology, College of Veterinary and Animal Sciences, Mannuthy, with the objective of evaluating the effects of induced stress (overcrowding by reducing the floor space by 50% so as to have a floor space of 348cm^2/bird) and three antistress agents (virginiamycin, probiotic and vitamin C) on some physiological and biochemical parameters, in both summer and rainy seasons. The experiment was conducted in four week old Vencob strain of broiler chicken for a period of four weeks.

3.1 EXPERIMENTAL BIRDS AND EXPERIMENTAL RATIONS

In each set of experiment, a total of 120, day-old broiler chicks of Vencob strain procured from a commercial hatchery were used for the study. The birds were reared in battery cages under standard managemental conditions upto four weeks of age. At fourth week of age the chicks were selected randomly, weighed, wing banded and divided into five experimental groups comprising of twelve birds in each group. Each experimental group was replicated twice. The details of the experimental groups of broiler chicken and the feed supplements given with their dosage are shown in table 1 and the unstressed control and stressed control groups of chicken are shown in figure 1.

The standard broiler rations (starter and finisher) were formulated as per the Bureau of Indian Standards specifications (BIS, 1992) and proximate principles were 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 from five to eight weeks of age. The ingredient composition and chemical composition of the above rations are presented in table 2, table 3.1 and table 3.2 respectively.

Table 1. Experimental groups of broiler chicken (n =12)

Groups	Stressor	Antistress agent	Remarks
G-I	Nil	Nil	Control (floor space 0.77 sq.ft/bird or 696 sq.cm/bird).
G-II	Overcrowding	Nil	Floor space reduced by 50 per cent (0.39 sq.ft/bird or 348 sq.cm/bird).
G-III	Overcrowding	Protexin soluble powder[1]	Floor space (0.39 sq.ft/bird) and supplemented with Protexin @ 0.025 per cent in diet
G-IV	Overcrowding	Virginiamycin[2]	Floor space (0.39 sq.ft/bird) and supplemented with Virginiamycin @ 0.002 per cent in diet
G-V	Overcrowding	Ascorbic acid[3]	Floor space (0.39 sq.ft/bird) and supplemented with ascorbic acid @ 0.03 per cent in diet

1. **Protexin soluble powder**- Probiotic with multiple strains of bacteria and yeast (1g of Protexin contains *Lactobacillus plantarum*- 1.26x10^8cfu, *Lactobacillus bulgaricus*-2.06x10^8cfu, *Lactobacillus acidophilus*–2.06x10^8cfu, *Lactobacillus casei*-2.06x10^8cfu, *Streptococcus thermophilus*-4.1x10^8cfu, *Streptococcus faecium*-5.4x10^8cfu, *Bifidobacterium bifidum*- 2x10^8cfu, *Torulopsis species*- 5.32x10^7cfu *Aspergillus oryzae*- 5.32x10^7cfu) – Novartis, Mumbai.
2. **Stafac – 20** - Virginiamycin 20g/kg – Pfizer, Mumbai.
3. **L-Ascorbic Acid (vitamin C) powder** - M/s. Sisco Research Laboratories Pvt. Ltd., Mumbai.

Fig. 1.1 Unstressed control group of broilers (G-I)

Fig. 1. 2 Stressed control group of broilers (G-II)

3.2 EXPERIMENT

The experiment was carried out in two phases.

Phase I – In summer season (March to May) 2004

Phase II – In rainy season (June to August) 2004

Ad libitum feed and water with identical photoperiod schedule were provided to all the birds throughout the experiment. Feed intake of birds was recorded replication wise at the end of each week and the average daily feed consumption per bird was calculated. The body weight of individual bird was recorded at weekly intervals from fourth to eighth week of age.

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

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

At eight weeks of age, the birds were put on fast for 10h. Each bird was weighed live; sacrificed by neck cut and allowed to bleed for 180s (Yalcin *et al.*, 1999). The birds were skinned and processed by removing the head, neck, shank and feet, eviscerated by cutting around the vent and carefully removing the viscera. The heart, liver and spleen were dissected from the viscera and weighed individually. The gizzard was cut open and rinsed off its contents with distilled water, dried and weighed. The dissected carcass of each bird along with its giblet was weighed. Carcass yield (dressing percentage) was calculated by expressing the dressed carcass weight as percentage of live body weight. The subcutaneous and abdominal fat pad as well as fat attached to the different organs in the body cavity was removed from the carcass using the method described by Karaoglu and Durdag (2005). The organ weight and fat content were expressed as percentage of live weight.

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.	Manganese sulphate (g)	10.00	10.00

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

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

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

Table 3.1 Proximate principles of broiler chicken ration

(On dry matter basis)

Phase – 1 (summer season)

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

* Mean of eight samples

Table 3.2 Proximate principles of broiler chicken ration

(On dry matter basis)

Phase – II (rainy season)

Sl. No.	Nutrients	Standard broiler ration (%)	
		Starter	Finisher
Analysed values*			
1.	Moisture	9.15	9.05
2.	Crude protein	22.96	19.95
3.	Ether extract	8.64	10.10
4.	Crude fibre	3.19	4.10
5.	Nitrogen free extract	53.75	53.88
6.	Total ash	11.46	11.97
7.	Acid insoluble ash	3.93	3.85
8.	Calcium	1.53	1.36
9.	Phosphorus	0.82	0.75
Calculated values			
10.	Metabolizable energy (kcal/kg)	2899.00	2981.00
11.	Lysine (%)	1.50	1.00
12.	Methionine (%)	0.53	0.40
13.	Manganese (mg/kg)	104.00	102.00

* Mean of eight samples

3.3 BLOOD COLLECTION

Blood samples were collected from six birds in each replicate from the wing vein with appropriate anticoagulants. Samples were collected initially at fourth week of age and then at fortnight intervals (at sixth and eighth week of age). From each bird, 6ml of blood was collected using ethylenediaminetetraaceticacid (EDTA) at the rate of 2mg/ml as the anticoagulant. Part of this blood was used for estimation of haematological parameters and the remaining blood was centrifuged at 3000rpm for 30min and the plasma was separated. 1ml of blood was collected using sodium fluoride (10mg/ml blood) as anticoagulant for plasma glucose estimation. 1ml of blood collected with heparin (20IU/ml blood) as anticoagulant, was used for mitogen induced lymphocyte proliferation test. Plasma samples were aliquoted and stored at –20°C for hormonal assay and at – 4°C for biochemical analysis. Haematological analysis and estimation of concentration of plasma enzymes were carried out on the day of collection.

3.4 HAEMATOLOGICAL PARAMETERS

Total erythrocyte count (TEC) and total leucocyte count (TLC) were estimated by the method suggested by Natt and Herrick (1952). Volume of packed red blood cells (VPRC) was estimated on the day of blood collection as per standard procedures (Feldman *et al.*, 2000). Concentration of haemoglobin (Hb) was determined using 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 glass slide using fresh blood at the time of blood collection. Air-dried smears were stained with Leishman-Giemsa stain solution and different leucocytes were counted and their percentages were calculated. Heterophil:Lymphocyte (H: L) ratio was also calculated (Gross and Siegel, 1983).

3.4.1 Leishman-Giemsa Staining

3.4.1.1 Preparation of the Stain

Leishman stain powder (0.15g) and Giemsa powder (0.03g) were ground with small amounts of acetone free methyl alcohol until an even suspension of stain was obtained. A total of 100 ml of acetone free methyl alcohol was added to produce a complete solution. The stain solution was filtered, poured into a dark bottle and aged for three weeks prior to use.

3.4.1.2 Method of Staining

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

3.4.2 Lymphocyte Blastogenic Response

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).

3.4.2.1 Principle

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

3.4.2.2 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 the pH was adjusted to 7.2 and filtered through 0.22μm cellulose acetate filter aseptically.
2. Bacto-Phytohaemagglutinin-M-1 per cent (PHA-M). Difco Laboratories, Detroit, Michigan, USA: Freeze dried powder reconstituted with 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 700mg of streptomycin (M/s Sarabhai Primal Pharmaceuticals Ltd., Vadodara) were incorporated per litre of medium.
5. Phosphate buffered saline (PBS - pH 7.2)
   A. 1.7g of KH_2PO_4 was dissolved in 250ml DDW
   B. 4.45g of $Na_2HPO_4 . 2H_2O$ was dissolved in 500ml DDW
   C. 250ml of A was mixed with 387.5ml of B to prepare PBS and the pH was adjusted to 7.2

3.4.2.3 Procedure

Three millilitre of heparinised blood was diluted two times with phosphate buffered saline (PBS - pH 7.2) and carefully layered over 3ml of ficol-paque solution in a centrifuge tube and centrifuged at 3000rpm for 20min. 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 10ml of PBS.

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

Seven millilitre of sterile RPMI-1640 medium was taken in a sterile culture vial into which 150µl PHA-M and 500µl 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 under study. Incubation was carried out for 72h at 37°C. At the end of 72h, lymphocyte number was determined haemocytometrically and the results were expressed as number of lymphocytes present per 500µl. The mitogenic response of lymphocyte number was compared with the control.

3.5 BIOCHEMICAL PARAMETERS

3.5.1 Plasma Protein profile

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

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

The plasma immunoglobulin G (IgG) level of pooled samples from each group was estimated in triplicate by single radial immunodiffusion (SRID) developed by Mancini *et al.* (1969) and modified by Nair (1990).

3.5.1.1 Quantification of Immunoglobulin G (IgG) by Radial Immunodiffusion

3.5.1.1.1 Reagents

1. P.B.S. (pH 7.3)

NaCl	8.00 g
K_2HPO_4	1.21 g
KH_2PO_4	0.34 g
Distilled water	1000 ml

2. 1.5 per cent agarose gel in P.B.S.
3. Antiserum to chicken IgG (purchased from Genei, Banglore)
4. Staining solution

Amido black 10B	1g
Sodium acetate- acetic acid buffer 0.2 M, pH 3.6	1000ml

5. Decolourising solutions for immunoelectrophoresis

5. a. Decolouriser I

Methanol	40 v
Acetic acid	10 v
Distilled water	10 v

5. b. Decolouriser II

Absolute alcohol	35 v
Acetic acid	5 v
Distilled water	10 v

3.5.1.1.2 Method

Agarose gel (1.5%) in PBS was melted and kept at 56°C in a water bath. Anti-serum against IgG warmed to 56°C, was added to the agarose gel to obtain a final concentration of 5 per cent of antiserum in the gel. Three millilitre of this mixture was over laid in agar-coated slides. After solidification, wells of 3mm diameter were punched out at a distance of 12mm between the wells. The wells were then charged with 10μl of varying dilutions of IgG of known protein concentration and incubated at 4°C for 24h in humid chamber.

Antigen-antibody precipitation rings formed around the wells were observed and the diameter was measured. The slides were washed, dried by overnight incubation at 37°C and immersed in staining solution for 15min. It is then decolourised by immersing in decolouriser I followed by decolouriser II (20min in each of the decolouriser). Duplicate determinations of the precipitation ring diameters were made and average values were taken to construct the standard curve, plotting the ring diameters against corresponding protein concentrations of antigen. This curve was used for calculation of IgG concentration in the test sample. The serum samples from the control and treatment group of broilers were used as the test samples.

3.5.1.2 Protein separation by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

3.5.1.2.1 Materials

3.5.1.2.1.1 Acrylamide-bisacrylamide stock (30:0.8)

Acrylamide	30.0g
Bisacrylamide	0.8g
Distilled water to make	100ml

Filtered through Whatman No.1 filter paper and stored at 4°C.

3.5.1.2.1.2 1.5 M Tris (pH 8.8)

Tris base	181.7 g
Distilled water to	1000 ml

Adjusted pH to 8.8 with 4N HCl and stored at 4°C.

3.5.1.2.1.3 0.5 M Tris

Tris base	60.6 g.
Distilled water to	1000 ml

Adjusted pH to 6.8 with 4 N HCl and stored at 4°C.

3.5.1.2.1.4 Resolving gel (12.5 per cent)

Acrylamide : bisacrylamide (30:0.8)	12.5 ml
Tris hydrochloride (1.5 M) pH 8.8	7.5 ml
Sodium dodecyl sulphate (10%)	0.3 ml
Ammonium persulphate (10%)	0.15 ml
N, N, N, N - tetra methyl ethylenediamine (TEMED)	0.01 ml
Distilled water	9.6 ml

3.5.1.2.1.5 Stacking gel (4%)

Acrylamide stock (30.8%)	0.67 ml
Tris hydrochloride (1.5 M) pH 6.8	1.25 ml
Sodium dodecyl sulphate (10%)	0.05 ml
Ammonium persulphate (10%)	25 µl
N, N, N, N - tetra methyl ethylenediamine (TEMED)	2.5 µl
Distilled water	3.00 ml

3.5.1.2.1.6 Electrophoresis buffer

Tris base	3.0 g
Glycine	14.4 g
Sodium dodecyl sulphate	1.0 g
Distilled water to make	1000 ml

3.5.1.2.1.7 Sample preparation buffer (2x)

0.5 M Tris hydrochloride, pH 6.8	2.5 ml
Glycerol	2.0 ml
Sodium dodecyl sulphate (ten per cent)	4.0 ml

2-mercaptoethanol	0.2 ml
Bromophenol blue	0.5 mg
Distilled water to make	10.0 ml

Distributed in small aliquots and stored at 4°C.

3.5.1.2.1.8 De-staining solution I

Glacial acetic acid	70 ml
Methanol	400 ml
Distilled water to make	1000 ml

3.5.1.2.1. 9 De-staining solution II

Glacial acetic acid	70 ml
Methanol	50 ml
Distilled water to make	1000 ml

3.5.1.2.1.10 Coomassie brilliant blue (SRL) staining solution

Coomassie brilliant blue (R250)	0.5 g
Methanol	800 ml
Glacial acetic acid	140 ml
Distilled water to	2000 ml

3.5.1.2.2 Method

The serum proteins of control and treatment group of birds were analysed by discontinuous system of polyacrylamide gel electrophoresis (Laemmli, 1970). Pooled samples (both sixth and eighth week) from control and treatment groups were used for the study.

Resolving gel solution (12.5 %) was prepared and degassed. Ten per cent ammonium persulphate and TEMED were added and poured between two glass plates so as to form a gel of 1mm thickness and left for polymerization. Distilled water was layered on the top to ensure uniformity of the gel surface. The set up was left overnight at 4°C for complete polymerization. After polymerization the distilled water was pipetted out and four per cent stacking gel was prepared and poured between the glass plates. The comb was then inserted and the apparatus left as such for complete polymerization for one and a half hours. After polymerization, the comb was removed and the wells were washed thoroughly with running buffer. A small quantity of running buffer was added into each of these wells. Twenty micrograms of the sample was mixed with 10μl of sample preparation buffer and kept in boiling water bath for five minutes. The samples were loaded into individual wells under the column of buffer in each well. The glass plates were fixed on to the vertical slab gel electrophoresis apparatus and electrophoresis buffer was carefully poured into the top and bottom reservoirs and electrophoresed at 10mA constant current till the bromophenol blue marker reached near the bottom of the resolving gel. The gel was then removed from the glass plate, the stacking gel was snipped off and transferred to a petri plate containing Coomassie brilliant blue staining solution and kept for two to three hours. The gels were then destained till the background became clear and were viewed in white light and photographed.

3.5.2 Plasma Lipid Profile

Concentration of plasma total lipids was estimated by Phosphovanilline method (Chabrol, 1961) using Labkit® kit (M/s. Labkit, Spain).

Concentration of plasma triglycerides was estimated by the enzymatic method (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 Liver Function Tests

3.5.4.1 Bile Pigments

Bilirubin content of the plasma was estimated photometrically by the method described by Alan (1988).

3.5.4.2 Plasma Enzymes

Plasma concentrations of enzymes such as Alanine amino transferase (ALT) and Aspartate amino transferase (AST) and Lactate dehydrogenase (LDH) were estimated by the photometric method described by Alan (1988).

3.6 HORMONAL ASSAY

3.6.1 Cortisol

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

3.6.2 Corticosterone

Concentration of plasma corticosterone was estimated by Enzyme immuno assay technique using Enzyme immuno assay kit (Assay Designs, Inc., Ann Arbor, U.S.A).

3.6.3 Thyroid Hormones (T_3 and T_4)

Tri-iodothyronine (T_3) and Thyroxine (T_4) concentrations in the plasma were estimated by RIA using gamma coat T_3 ^{125}I RIA commercial kit (M/s. Diasorin, Minnesota, U.S.A) and gamma coat T_4 ^{125}I RIA commercial kit (M/s. Diasorin, Minnesota, U.S.A) respectively.

3.7 CLIMATIC DATA

Throughout the period of study, the maximum and minimum temperatures and relative humidity (R.H) in the animal room (where the birds were reared) were recorded using maximum and minimum thermometer and hygrometer respectively. The data regarding the rainfall during the period of study was obtained from Kerala Agricultural University observatory.

3.8 ECONOMICS OF PRODUCTION

The average cost of production and total returns from a bird at eighth week of age was calculated to assess the cost benefit ratio. The cost of production included the cost of chick, cost of starter and finisher rations and miscellaneous cost. The miscellaneous cost includes the cost of vaccinations, electricity and labour cost. Since the experiment was started at four weeks of age, the cost of starter ration for all the groups were considered as equal. Economics of production was calculated by subtracting the expenditure for each group from the total income.

3.9 STATISTICAL ANALYSIS

Data collected on various parameters were statistically analyzed as per the method of Snedecor and Cochran (1989) by employing student's 't' test when only two groups were compared while one way analysis of variance (ANOVA) followed by least significant difference (LSD) test adopted when more than one group were compared the indices for test of significance in the above mentioned statistical tests used were the t value and the calculated value of F (CVF) respectively.

The completely randomized design (CRD) was employed in split time plot experiment when test for significance included more than one group simultaneously at different time periods. The critical difference (CD) value was used to find out the level of significant difference within groups and between groups.

Results

4. RESULTS

4.1 CLIMATIC DATA

The present study was conducted in summer (March - May) and rainy (June - August) seasons. The average weekly temperature, humidity and rainfall recorded during the experimental period are presented in table 4.1 and 4.2. The overall mean temperature (maximum and minimum), relative humidity (RH) and rainfall to which the birds were exposed before (1 to 4 weeks) and after (4 to 8 weeks) commencing the experiment are also shown in table 4.1 and 4.2.

In summer, before start of the experiment (during the first four weeks of age) the birds were exposed to an average maximum temperature of 34.68±0.30°C and minimum temperature of 25.03±0.20°C and the RH from 53.35±1.10 to 85.03±0.90 per cent. During the period 0.88±0.50mm rainfall was recorded. In the rainy season the maximum temperature recorded was 29.93±0.20°C and minimum temperature was 23.25±0.10°C. The RH varied from 73.80±0.10 to 92.75±0.20 per cent. A rainfall of 13.45±2.60mm was also recorded during this period.

After inducing stress through overcrowding (from 5 to 8 weeks) the birds were exposed to an average maximum temperature of 32.68±1.17°C and minimum temperature of 24.38±0.46°C in summer season. The rainfall recorded was 13.50±5.19mm. The RH during the period ranged from 64.55±5.62 to 88.25±1.71 per cent. In the rainy season the maximum temperature was 28.93±0.26°C and minimum was 22.83±0.12°C. The RH during the period ranged from 77.85±1.40 to 93.85±0.22 per cent. The rainfall recorded during this period was19.03±5.21mm.

Table 4.1 Weekly meteorological data during the experimental period of eight weeks*

Summer season

Week	**Temperature (°C)**		**Relative Humidity (%) (RH)**		**Rainfall (mm)**
	Maximum	Minimum	Forenoon	Afternoon	
I	34.70±0.30	25.40±0.10	86.90±1.00	51.10±2.30	0
II	35.10±0.30	24.00±0.40	86.40±2.40	52.60±1.40	2.20±1.20
III	33.80±0.90	24.60±0.60	84.90±1.90	55.70±3.40	1.30±1.30
IV	35.10±0.30	26.10±0.30	81.90±1.40	54.00±1.40	0
V	35.00±0.60	25.20±0.50	86.90±1.00	52.90±2.30	4.90±3.90
VI	34.80±0.30	24.80±0.40	84.90±1.90	55.00±2.50	12.30±10.80
VII	29.30±0.90	23.50±0.20	91.60±1.70	81.90±3.90	30.00±10.70
VIII	31.60±0.40	24.00±0.40	89.60±1.78	68.40±4.67	6.80±3.22
[1]Mean ±SE	34.68 ±0.30	25.03±0.20	85.03±0.90	53.35 ±1.10	0.88 ±0.50
[2]Mean ±SE	32.68±1.17	24.38 ±0.46	88.25±1.71	64.55±5.62	13.50±5.19
[3]Mean ±SE	33.68 ±0.75	24.70 ±0.30	86.64 ±1.05	58.95 ±3.79	7.19±3.57

* From 22/3/04 to17/5/04

1 Mean values of I to IV weeks

2 Mean values of V to VIII weeks

3 Mean values of I to VIII weeks

Table 4.2 Weekly meteorological data during the experimental period of eight weeks*

Rainy Season

Week	Temperature (°C)		Relative Humidity (%) (RH)		Rainfall (mm)
	Maximum	Minimum	Forenoon	Afternoon	
I	29.30±0.20	23.30±0.20	92.40±0.20	80.60±0.20	25.10±3.90
II	30.80±0.20	23.40±0.20	92.60±0.20	66.30±0.20	2.50±1.40
III	29.70±0.40	22.90±0.20	92.40±0.40	73.40±0.20	19.50±6.30
IV	29.90±0.40	23.40±0.20	93.60±0.40	74.90±0.20	6.70±2.70
V	28.60±0.60	22.90±0.30	93.10±0.60	78.10±0.30	11.90±6.50
VI	29.50±0.40	22.80±1.00	94.40±0.40	74.70±1.00	8.00±3.90
VII	29.00±0.40	22.90±0.20	93.90±0.40	76.30±0.20	21.60±5.30
VIII	28.60±0.75	22.70±0.34	94.00±0.75	82.30±0.34	34.60±17.35
[1]Mean ±SE	29.93±0.20	23.25±0.10	92.75±0.20	73.80±0.10	13.45±2.60
[2]Mean ±SE	28.93±0.26	22.83±0.12	93.85±0.22	77.85±1.40	19.03±5.21
[3]Mean ±SE	29.43 ±0.26	23.04 ±0.10	93.30±0.29	75.83±1.74	16.24±3.83

* From11/6/04 to 5/8/04

1 Mean value of I to IV weeks

2 Mean value of V to VIII weeks

3 Mean value of I to VIII weeks

4.2 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON HAEMATOLOGICAL PARAMETERS

4.2.1 Total Erythrocyte Count (TEC)

Total erythrocyte count of control and treatment groups of birds are presented in table 5.1. During summer and rainy seasons, no significant ($P>0.05$) difference in the TEC value was observed initially (at fourth week of age) among different groups of broiler chicken. In the summer season, the value of TEC in four week old broilers ranged from $2.81 \pm 0.07 x 10^6/\mu l$ to $2.90 \pm 0.07 x 10^6/\mu l$. The highest ($2.90 \pm 0.07 x 10^6/\mu l$) erythrocyte count was observed in stressed control G-II group. The lowest value ($2.81 \pm 0.07 x 10^6/\mu l$) was in G-III (overcrowded birds supplemented with Protexin) group of birds. In the rainy season, the TEC value of four week old birds was highest ($3.03 \pm 0.05 x 10^6/\mu l$) in G-III group of birds and lowest ($2.96 \pm 0.04 x 10^6/\mu l$) in G-I group of birds.

At six week of age (14 days after overcrowding stress), no significant difference in TEC was observed between groups and between seasons. In summer, the TEC value of six week old birds ranged from $2.93 \pm 0.04 x 10^6/\mu l$ in G-IV (overcrowded birds supplemented with virginamycin) to $2.99 \pm 0.06 x 10^6/\mu l$ in G-III group of birds. During the rainy season, among the six week old birds the highest TEC value ($3.05 \pm 0.04 x 10^6/\mu l$) was seen in birds of G-III group and lowest ($2.98 \pm 0.04 x 10^6/\mu l$) in birds of G-IV group. The investigations revealed that, antibiotic (virginamycin) supplementation during summer and rainy seasons produced a non-significant reduction in the value of TEC in six week old overcrowded birds.

In summer, eight week old stressed birds supplemented with vitamin C (G-V) had a significantly ($P \leq 0.05$) higher TEC value ($3.08 \pm 0.04 x 10^6/\mu l$) when compared to the value in the virginiamycin supplemented G-IV group of birds ($2.94 \pm 0.04 x 10^6/\mu l$). A significant ($P \leq 0.05$) increase in TEC value was noticed between periods (4 and 8 weeks) in G-I, G-II and G-III groups of birds during the summer season whereas in G-IV group of birds the increase was non-significant.

The birds of G-V (overcrowded birds supplemented with vitamin C) had a significant ($P \leq 0.05$) increase in TEC value with advancement of age (Table 5.1). Similar to the observations in the summer season, in the rainy season also, the TEC value of eight week old birds in G-V group showed a significant ($P \leq 0.05$) increase when compared to TEC value of G-IV group of birds. The highest TEC value ($3.09 \pm 0.04 \times 10^6/\mu l$) was noted in birds of G-V group and the lowest ($2.97 \pm 0.04 \times 10^6/\mu l$) in birds of G-IV group.

4.2.2 Haemoglobin (Hb) Concentration

Table 5.1 presents the Hb concentration of control and treatment groups of broiler chicks at fourth, sixth and eighth week of age in summer and rainy seasons. The Hb concentration of birds at 4th week of age before applying the overcrowding stress between different groups under study did not differ significantly ($P>0.05$) in summer or in rainy season. In birds of G-I, G-IV and G-V groups the Hb concentration was significantly ($P \leq 0.05$) lower in the summer, when compared to those in the rainy season.

In four week old birds of summer season the highest Hb concentration (8.96±0.49g%) was noted in G-IV group of birds and lowest (8.54±0.48g%) value in G-I group of birds. In rainy season, the Hb content of four week old birds ranged from 9.81±0.14g per cent in G-III group of birds to 10.25±0.30g per cent in G-IV group of birds respectively.

The concentration of Hb was not altered by floor space reduction (348cm^2/ bird) stress in six week old birds in both phases of the study (summer and rainy seasons). The Protexin, virginiamycin and vitamin C used for the study did not significantly ($P>0.05$) affect the Hb content of the stressed birds. As far as phase I is concerned, maximum concentration of Hb (9.58±0.38g %) was noticed in birds of G-V group (overcrowded birds supplemented with vitamin C) and minimum (8.79±0.30g%) in birds of G-IV group (overcrowded birds supplemented with virginiamycin). In the rainy season, the Hb concentration varied from 9.68±0.12 g per cent in birds of G-II group (stressed control) to 10.13±0.25g per cent in birds of G-V group.

In G-III (overcrowded birds supplemented with Protexin) and G-IV (overcrowded birds supplemented with virginiamycin) groups of birds, a significantly ($P \leq 0.05$) reduced Hb level noticed in the summer when compared to their counterparts in the rainy season. Compared to the birds in the rainy season a decrease (non-significant) in the Hb value was observed in all groups of birds during the summer irrespective of the applied stress and supplementation of antistress agents.

At eighth week of age, no significant difference in the Hb concentration was observed between different groups of birds in summer. However, in the rainy season significantly ($P \leq 0.05$) reduced Hb level (9.74±0.37g%) was noticed in virginiamycin supplemented stressed birds (G-IV) when compared to G-I (unstressed control) and G-V (overcrowded birds supplemented with vitamin C) group of birds.

In summer, Hb content of eight week old birds ranged from 8.83±0.41g per cent in birds of G-IV group to 9.75±0.51g per cent in birds of G-I group. In the rainy season the mean Hb value ranged from 9.74±0.37g per cent in birds of G-IV group to 10.38±0.13g per cent in birds of G-I group.

On comparison of Hb concentration of eight week old broilers of summer and rainy seasons it was seen that there was a significant ($P \leq 0.05$) decrease in Hb level in the summer in G-II and G-III group of birds. In birds of all groups, the Hb level was lower in summer than in the rainy season.

Table 5.1 Effect of overcrowding stress and antistress agents on haematological parameters of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameter		TEC x $10^6/\mu l$			Hb (g %)			VPRC (%)		
Seasons	Weeks / Groups	4	6	8	4	6	8	4	6	8
S_1	G -I	$2.88\pm0.04^{ax}_{NS}$	$2.95\pm0.06^{axy}_{NS}$	$2.97\pm0.04^{aby}_{NS}$	$8.54\pm0.48^{ax**}$	9.29 ± 0.49^{axy}	9.75 ± 0.51^{ay}	$27.42\pm0.63^{ax**}$	29.50 ± 0.56^{ay}	29.33 ± 0.79^{ay}
	G -II	$2.90\pm0.07^{ax}_{NS}$	$2.99\pm0.02^{axy}_{NS}$	$2.97\pm0.04^{aby}_{NS}$	8.83 ± 0.52^{ax}	9.13 ± 0.38^{ax}	$9.21\pm0.40^{ax*}$	$27.33\pm0.72^{ax**}$	28.25 ± 0.63^{abx}	$27.83\pm0.58^{abx**}$
	G -III	$2.81\pm0.07^{ax}_{NS}$	$2.99\pm0.06^{axy}_{NS}$	$3.01\pm0.04^{aby}_{NS}$	8.88 ± 0.51^{ax}	$9.08\pm0.39^{ax*}$	$9.17\pm0.33^{ax*}$	$27.67\pm0.73^{ax**}$	29.25 ± 0.63^{ay}	29.42 ± 0.63^{ay}
	G -IV	$2.90\pm0.04^{ax}_{NS}$	$2.93\pm0.04^{ax}_{NS}$	$2.94\pm0.04^{ax}_{NS}$	$8.96\pm0.49^{ax*}$	$8.79\pm0.30^{ax*}$	8.83 ± 0.41^{ax}	$27.83\pm0.67^{ax**}$	$27.17\pm0.58^{bx*}$	$27.33\pm0.71^{bx**}$
	G -V	$2.89\pm0.04^{ax}_{NS}$	$2.97\pm0.04^{ay}_{NS}$	$3.08\pm0.04^{bz}_{NS}$	$8.92\pm0.42^{ax**}$	9.58 ± 0.38^{ax}	9.67 ± 0.42^{ax}	$27.25\pm0.71^{ax**}$	28.92 ± 0.87^{ay}	29.25 ± 0.63^{ay}
S_2	G -I	$2.96\pm0.04^{ax}_{NS}$	$3.02\pm0.04^{ax}_{NS}$	$3.02\pm0.06^{abx}_{NS}$	$10.12\pm0.20^{ax**}$	9.79 ± 0.13^{ax}	10.38 ± 0.13^{ax}	$30.00\pm0.21^{ax**}$	30.42 ± 0.36^{ax}	30.25 ± 0.35^{ax}
	G -II	$2.98\pm0.06^{ax}_{NS}$	$3.04\pm0.03^{ax}_{NS}$	$3.00\pm0.03^{abx}_{NS}$	9.83 ± 0.26^{ax}	9.68 ± 0.12^{ax}	$10.19\pm0.28^{abx*}$	$30.25\pm0.30^{ax**}$	29.97 ± 0.44^{abx}	$30.05\pm0.35^{ax**}$
	G -III	$3.03\pm0.05^{ax}_{NS}$	$3.05\pm0.04^{ax}_{NS}$	$3.03\pm0.04^{abx}_{NS}$	9.81 ± 0.14^{ax}	$10.00\pm0.11^{ax*}$	$10.17\pm0.17^{abx*}$	$30.42\pm0.50^{ax**}$	30.25 ± 0.79^{ax}	30.33 ± 0.26^{ax}
	G -IV	$3.00\pm0.05^{ax}_{NS}$	$2.98\pm0.04^{ax}_{NS}$	$2.97\pm0.04^{ax}_{NS}$	$10.25\pm0.30^{ax*}$	$9.83\pm0.32^{ax*}$	9.74 ± 0.37^{bx}	$30.33\pm0.22^{ax**}$	$29.33\pm0.53^{bx*}$	$30.17\pm0.32^{ax**}$
	G -V	$2.99\pm0.06^{ax}_{NS}$	$2.99\pm0.04^{ax}_{NS}$	$3.09\pm0.04^{bx}_{NS}$	$10.17\pm0.14^{ax**}$	10.13 ± 0.25^{ax}	10.21 ± 0.26^{ax}	$29.83\pm0.24^{ax**}$	30.67 ± 0.26^{ax}	30.42 ± 0.45^{ax}

G -I Unstressed control (floor space / bird = $696cm^2$), **G -II** Overcrowded control (floor space / bird = $348cm^2$), **G -III** Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and **G -V** Overcrowded and vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

a; b Means within a column with no common superscripts are significantly different at 5% level

x, y, z Means within a row with no common superscripts are significantly different at 5% level

* Between seasons significantly different at 5% level

** Between seasons significantly different at 1% level

NS No significant difference between seasons s_1 and s_2

TEC Total erythrocyte count. **Hb** Haemoglobin. **VPRC** Volume of packed red blood cells

4.2.3 Volume of Packed Red Blood Cells (VPRC)

The effect of overcrowding stress (floor space reduction of 50%) and antistress agents (Protexin, virginiamycin and vitamin C) on VPRC value in broilers at sixth and eighth weeks of age in summer and rainy seasons are depicted in the table 5.1.

The value of VPRC in birds at fourth week of age showed no significant difference between groups in both summer and rainy seasons. However, a highly significant (P≤0.01) difference in the VPRC value was noticed between the birds in the two seasons with the values in summer being lower than those in the rainy season. In the summer season, the VPRC level in four week old birds ranged from 27.25±0.71 per cent in birds of G-V group to 27.83±0.67 per cent in birds of G-IV group. In the rainy season, maximum VPRC value of 30.42±0.50 per cent was noticed in birds of G-III group and minimum value of 29.83±0.24 per cent in birds of G-V group.

On comparing the VPRC values of four week old broilers reared in summer and rainy seasons, a highly significant (P≤0.01) reduction in the value of VPRC was noted in all groups of birds in the summer vide table 5.1.

In summer (March to May), among the different groups of birds at sixth week of age, the VPRC value was significantly (P≤0.05) low in overcrowded birds fed with virginiamycin (27.17±0.58%) when compared to unstressed control birds (G-I), overcrowded birds supplemented with Protexin (G-III) and overcrowded birds supplemented with vitamin C (G-V). But there was no significant (P>0.05) difference in the haematocrit value between unstressed control (G-I), stressed control (G-II), overcrowded birds supplemented with Protexin (G-III) and stressed birds supplemented with vitamin C (G-V). The maximum VPRC level (29.50±0.56%) was noticed in G-I group of birds and minimum value (27.17±0.58%) was observed in G-IV group of birds.

Similar to the observations in summer, in the rainy season a significant ($P \leq 0.05$) reduction in the VPRC value was observed in overcrowded birds supplemented with virginiamycin (G-IV) at sixth week when compared to the birds of G-I, G-III and G-V groups. The highest VPRC value (30.67±0.26%) was observed in G-V group of birds. The birds in G-IV group showed the lowest VPRC value (29.33±0.53%).

Among the different groups of birds at sixth week of age no significant ($P > 0.05$) difference in the value of VPRC was observed between summer and rainy seasons except in the G-IV group of birds. In this group the VPRC level was significantly ($P \leq 0.05$) lower in summer (27.17±0.58%) than in rainy season (29.33±0.53%). Even though there was no significant difference in the VPRC value between summer and rainy seasons in G-I, G-II, G-III and G-V group of birds, a numerically lower VPRC level was noticed in birds reared in the summer than their counterparts in the rainy season.

In eight week old birds reared in the summer months (April and May) a significantly ($P \leq 0.05$) lower VPRC value (27.33±0.71%) was observed in birds of G-IV group when compared to that of G-I, G-III and G-V groups of birds vide table 5.1.

The highest value of VPRC (29.42±0.63%) was noticed in stressed birds supplemented with Protexin (G-III) and lowest (27.33±0.71%) level in overcrowded birds supplemented with virginiamycin (G- IV).

In spite of the stress applied and antistress agents supplemented no significant change in VPRC value was observed among the eight week old birds of different groups in rainy season. The lowest value (30.05±0.35%) was in birds of G-II group and the highest (30.42±0.45%) in birds of G-V group. Analysis of seasonal effect on haematocrit in control and treatment groups of birds revealed a highly significant ($P \leq 0.01$) reduction in VPRC levels in G-II and G-IV groups of birds in summer. Uniformly low VPRC value was seen in all birds during summer, when compared to that of rainy season vide table 5.1.

In summer the VPRC values in unstressed control birds (G-I), overcrowded birds supplemented with Protexin (G-III) and overcrowded birds supplemented with vitamin C (G-V) increased significantly ($P \leq 0.05$) between four and six weeks as well as four and eight weeks of age. But this increase was not observed in the rainy season. In G-II and G-IV group of birds the differences in VPRC value between ages were not significant ($P > 0.05$) either in summer or in rainy season (Table 5.1).

4.2.4 Erythrocyte Indices

4.2.4.1 Mean Corpuscular Volume (MCV)

The MCV values of control and treatment groups of birds in summer and rainy seasons are depicted in table 5.2.

In summer, the value of MCV at fourth week of age ranged from 94.63±3.18fl in G-V group of birds to 96.78±4.11fl in G-III group of birds. In rainy season, the MCV values of four week old chicks ranged from 99.78±1.96fl in G-IV to 101.72±2.43fl in G-II group of birds. There was no significant ($P > 0.05$) difference in MCV value between the birds in different groups vide table 5.2.

On comparing the MCV values of birds in summer and rainy seasons no significant ($P > 0.05$) difference in the MCV value was noticed between the different groups except in G-I and G-II groups of birds. In control G-I and stressed control G-II groups of birds the MCV value was significantly ($P \leq 0.05$) low in summer when compared to those in rainy season. A uniformly low value of MCV was obtained in birds during the summer season.

Table 5.2 Effect of overcrowding stress and antistress agents on haematological parameters (Erythrocyte indices) of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameters		MCV (fl)			MCH (pg)			MCHC (g%)		
Seasons	Weeks / Groups	4	6	8	4	6	8	4	6	8
S_1	G -I	95.37±2.40^{ax}*	100.34±2.76^{ax}	98.85±2.86^{ax}	29.67±1.62^{ax}	31.50±1.58^{axy}	32.91±1.80^{ay}	31.46±2.08$^{ax}_{NS}$	31.73±1.92$^{ax}_{NS}$	33.24±1.57$^{ax}_{NS}$
	G -II	94.70±3.43^{ax}*	94.58±2.23^{abx}	93.98±2.4^{ax}	30.55±1.80^{ax}	32.24±1.33^{ax}	30.99±1.22^{ax}	32.80±2.43$^{ax}_{NS}$	34.24±1.48$^{ax}_{NS}$	33.09±1.32$^{ax}_{NS}$
	G -III	96.78±4.11^{ax}	98.36±2.71^{abx}	97.85±2.11^{ax}	31.07±2.05^{ax}	30.63±1.59^{ax}	30.55±1.24^{ax}	32.31±2.03$^{ax}_{NS}$	31.34±1.70$^{ax}_{NS}$	31.63±1.18$^{ax}_{NS}$
	G -IV	95.93±2.14^{ax}	92.41±2.11^{bx}	93.29±2.81^{ax}*	30.94±1.78^{ax}	29.96±1.15^{ax}	30.24±1.63^{ax}	32.43±2.02$^{ax}_{NS}$	32.49±1.25$^{ax}_{NS}$	32.77±1.03$^{ax}_{NS}$
	G -V	94.63±3.18^{ax}	97.72±3.67^{abx}	95.27±2.63^{ax}	30.95±1.58^{ax}	32.29±1.28^{ax}	31.35±1.20^{ax}	32.83±1.42$^{ax}_{NS}$	33.34±1.37$^{ax}_{NS}$	33.26±1.65$^{ax}_{NS}$
S_2	G -I	101.40±1.06^{ax}*	100.94±1.61^{ax}	101.43±1.99^{ax}	33.23±0.74^{ax}	33.51±0.65^{ax}	34.79±0.67^{ax}	33.75±1.61$^{ax}_{NS}$	33.22±1.47$^{ax}_{NS}$	34.31±1.33$^{ax}_{NS}$
	G -II	101.72±2.43^{ax}*	97.70±1.89^{ax}	99.20±1.32^{ax}	33.09±0.76^{ax}	31.68±0.51^{ax}	33.01±1.03^{ax}	32.55±0.95$^{ax}_{NS}$	33.52±0.98$^{ax}_{NS}$	33.32±1.10$^{ax}_{NS}$
	G -III	100.64±2.20^{ax}	98.84±2.79^{ax}	101.50±1.21^{ax}	32.12±0.68^{ax}	32.70±0.63^{ax}	34.08±0.88^{ax}	31.96±1.44$^{ax}_{NS}$	33.32±0.97$^{ax}_{NS}$	33.54±1.60$^{ax}_{NS}$
	G -IV	99.78±1.96^{ax}	98.67±2.50^{ax}	101.81±1.68^{ax}*	33.72±1.14^{ax}	33.11±1.29^{ax}	32.74±1.21^{ax}	33.83±1.06$^{ax}_{NS}$	32.52±0.87$^{ax}_{NS}$	32.23±1.28$^{ax}_{NS}$
	G -V	100.10±2.13^{ax}	101.08±1.37^{ax}	100.42±1.55^{ax}	33.14±0.93^{ax}	33.64±0.78^{ax}	33.05±0.93^{ax}	34.10±1.53$^{ax}_{NS}$	33.96±0.91$^{ax}_{NS}$	33.69±1.13$^{ax}_{NS}$

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2), **G -III** Overcrowded and Protexin supplemented group, G -IV Overcrowded and virginiamycin supplemented group and **G -V** Overcrowded and vitamin C supplemented group

a, b Means within a column with no common superscripts are significantly different at 5% level

x, y Means within a row with no common superscripts are significantly different at 5% level

*- Between seasons significantly different at 5 % level

NS No significant difference between seasons s_1 and s_2

MCV Mean corpuscular volume. **MCH** Mean corpuscular haemoglobin. MCHC Mean corpuscular haemoglobin concentration

The value of MCV in six week old birds of summer ranged from 92.41±2.11fl in G-IV (overcrowded birds supplemented with virginiamycin) group of birds to 100.34±2.76fl in G-I (control) group of birds. Significantly ($P \le 0.05$) lower MCV value (92.41±2.11fl) was found in G-IV group of birds when compared to the value in birds of G-I group. The stressed control group of birds also had a low (nonsignificant) value (94.58±2.23fl) for MCV. In the rainy season, the MCV value of birds varied from 97.70±1.89fl in G-II (stressed control) group of birds to 101.08±1.37fl in G-V (overcrowded birds supplemented with vitamin C) group of birds. No significant change in the MCV value was noticed among birds of different groups. The overcrowding produced a lower MCV value, which was not significant ($P>0.05$). The value of MCV in virginiamycin and probiotic supplemented stressed birds (G-IV and G-III group respectively) were also lower than that of control (G-I) birds.

The MCV value of birds in both summer and rainy seasons had no significant ($P>0.05$) difference in all the groups. Whereas, a uniformly low value was noticed in all the groups during the summer season.

In summer, the eight week old birds of G-IV group had the lowest (93.29±2.81fl) and that of G-I group had the highest MCV (98.85±2.86fl) value. In the second phase, the value of MCV ranged between 99.20±1.32fl in birds of G-II group and 101.81±1.68fl in G-IV group.

Eight week old birds of G-IV group had a significantly ($P \le 0.05$) low MCV value in the summer, when compared to their counterparts in the rainy season. The results of the experiment showed that, overcrowding produced reduction in MCV value in both summer and rainy seasons, which was not significant. In the summer probiotic supplementation was found to have maximum beneficial effect and virginiamycin had an adverse effect on the MCV values.

In rainy season, virginiamycin, Protexin and vitamin C were found to be equally effective in improving the MCV value in overcrowded birds at sixth and eighth week of age. Irrespective of the groups or ages the value of MCV was lower in summer than in the rainy season (Table 5.2). No significant difference in the MCV value was noticed with age (4-8 weeks) both in summer and rainy seasons.

4.2.4.2 Mean Corpuscular Haemoglobin (MCH)

The effect of overcrowding stress and antistress agents on the MCH value of six and eight weeks old broiler chicken in the summer as well as rainy seasons are depicted in table 5.2.

In the summer, the initial value of MCH (4th week of age) ranged from 29.67±1.62pg in G-I (unstressed control) group of birds to 31.07±2.05pg in G-III (overcrowded birds supplemented with Protexin) group of birds. In the rainy season the MCH value ranged from 32.12±0.68pg to 33.72±1.14pg. The lowest value of 32.12±0.68pg was noticed in G-III group of birds and highest value of 33.72±1.14pg in G-IV (overcrowded birds supplemented with virginiamycin) group of birds. No significant (P>0.05) difference in MCH value was observed between summer and rainy seasons.

Among the six week old birds of summer the highest MCH value (32.29±1.28pg) was observed in G-V (overcrowded birds supplemented with vitamin C) group of birds and lowest value (29.96±1.15pg) was observed in G-IV (overcrowded birds supplemented with virginiamycin) group of birds. In the rainy season the value was maximum (33.64±0.78pg) in birds of G-V group and minimum (31.68±0.51pg) in birds of G-II group. No significant change in the MCH value observed between four and six weeks old birds in any of the groups studied.

Among the eight week old birds in the summer, the MCH value was maximum (32.91±1.80pg) in G-I group of birds and minimum (30.24±1.63pg) in G-IV group of birds. In the rainy season also, the maximum value of

34.79±0.67pg was noticed in G-I group of birds and the minimum value of 32.74±1.21pg was noticed in G-IV group of birds. No significant change in the MCH value observed between six and eight weeks old birds in any of the groups studied.

In no groups a significant difference in MCH value was noticed between the seasons. However, the birds reared in the summer season showed a numerically lower value than those reared in the rainy season except the values in G-II group of birds at sixth week of age.

4.2.4.3 Mean Corpuscular Haemoglobin Concentration (MCHC)

Mean corpuscular haemoglobin concentration values of control (G-I and G-II) and treatment groups (G-III, G-IV, and G-V) of birds at fourth, sixth and eighth week of age in summer and rainy season are depicted in table 5.2.

In the summer the MCHC values before the application of stress (fourth week of age) varied from 31.46±2.08g per cent in control G-I group of birds to 32.83±1.42g per cent in G-V (overcrowded birds supplemented with vitamin C) group of birds. No significant (P>0.05) difference in MCHC value was noticed between the groups. In the rainy season the initial value of MCHC in four week old broiler chicks ranged between 31.96±1.44g per cent in G-III group of birds and 34.10±1.53g per cent in G-V group of birds.

The MCHC values noticed in six week old birds in the summer ranged from 31.34±1.70g per cent in G-III (overcrowded birds supplemented with Protexin) group of birds to 34.24±1.48g per cent in G-II (stressed control) group of birds. In the rainy season the MCHC values ranged from 32.52±0.87g per cent in G-IV (overcrowded birds supplemented with virginiamycin) group of birds to 33.96±0.91g per cent in G-V (overcrowded birds supplemented with vitamin C) group of birds. During both summer and rainy seasons there was no significant (P>0.05) difference in MCHC values between the control G-I group and the other groups of birds (G-II, G-III, G-IV, and G-V). No significant (P>0.05) difference in the values of MCHC was noticed between four and six weeks old birds of both phases of the experiment. Seasonal comparison of results also showed no significant (P>0.05) difference in the value of MCHC between summer and rainy seasons.

In the summer, among eight week old birds, the highest MCHC value of 33.26±1.65g per cent was observed in G-V group of birds and lowest MCHC value of 31.63±1.18g per cent was noticed in G-III group of birds. There was no significant (P>0.05) difference in the value of MCHC between the control G-I group of birds and treatment group of birds. Also, no significant (P>0.05) difference in the MCHC value was observed between the different age groups (4 and 6, 4 and 8, 6 and 8). In the rainy season, the value of MCHC in eight week old birds ranged from 32.23±1.28g per cent in G-IV (overcrowded birds supplemented with virginiamycin) group of birds to 34.31±1.33g per cent in control G-I group of birds. There was no significant (P>0.05) difference in the values of MCHC between birds of different age groups.

4.2.5 Total Leukocyte Count (TLC)

The effect of the overcrowding stress and antistress feed supplements (Protexin, virginiamycin and vitamin C) on the leukocyte count of six and eight weeks old broiler chicks in summer and rainy seasons are presented in table 5.3. Analysis of the data revealed no significant (P>0.05) difference in the value of TLC between groups, ages and seasons.

Table 5.3 Effect of overcrowding stress and antistress agents on haematological parameter (total leukocyte count) of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Seasons	Parameter / Weeks / Groups	TLC x $10^3/\mu l$ 4	6	8
S_1	G -I	$25.67 \pm 1.49^{ax}_{NS}$	$25.17 \pm 1.62^{ax}_{NS}$	$26.83 \pm 1.27^{ax}_{NS}$
	G -II	$25.83 \pm 1.51^{ax}_{NS}$	$25.17 \pm 1.14^{ax}_{NS}$	$24.83 \pm 1.19^{ax}_{NS}$
	G -III	$25.67 \pm 1.25^{ax}_{NS}$	$25.33 \pm 1.38^{ax}_{NS}$	$25.08 \pm 1.52^{ax}_{NS}$
	G -IV	$26.00 \pm 1.26^{ax}_{NS}$	$25.17 \pm 1.22^{ax}_{NS}$	$24.83 \pm 1.38^{ax}_{NS}$
	G -V	$25.50 \pm 1.44^{ax}_{NS}$	$25.67 \pm 1.25^{ax}_{NS}$	$26.75 \pm 0.66^{ax}_{NS}$
S_2	G -I	$27.33 \pm 1.48^{ax}_{NS}$	$27.67 \pm 1.61^{ax}_{NS}$	$28.17 \pm 1.60^{ax}_{NS}$
	G -II	$27.17 \pm 1.24^{ax}_{NS}$	$25.67 \pm 1.51^{ax}_{NS}$	$26.00 \pm 1.67^{ax}_{NS}$
	G -III	$28.50 \pm 1.64^{ax}_{NS}$	$27.33 \pm 1.66^{ax}_{NS}$	$28.00 \pm 1.58^{ax}_{NS}$
	G -IV	$27.00 \pm 1.53^{ax}_{NS}$	$26.67 \pm 1.60^{ax}_{NS}$	$26.50 \pm 1.37^{ax}_{NS}$
	G -V	$26.83 \pm 1.71^{ax}_{NS}$	$27.00 \pm 1.03^{ax}_{NS}$	$26.67 \pm 1.52^{ax}_{NS}$

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2), **G -III** Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and **G -V** Overcrowded and vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

Means within a column with common superscript did not differ significantly at 5% level

Means within a row with common superscript did not differ significantly at 5% level

NS No significant difference between seasons s_1 and s_2

TLC Total leukocyte count.

In the summer season the TLC value of four week old birds (before applying overcrowding stress) ranged from $25.50 \pm 1.44 \times 10^3/\mu l$ in G-V group to $26.00 \pm 1.26 \times 10^3/\mu l$ in G-IV group of birds. In the rainy season, the value varied from $26.83 \pm 1.71 \times 10^3/\mu l$ in birds of G-V group to $28.50 \pm 1.64 \times 10^3/\mu l$ in birds of G-III group. Even though, there was no significant ($P>0.05$) difference in TLC value of birds in the two seasons, the count was lower in the summer compared to the values in the rainy season. The TLC value was highest ($28.50 \pm 1.64 \times 10^3/\mu l$) in birds of G-III group in the rainy season and lowest ($25.50 \pm 1.44 \times 10^3/\mu l$) in birds of G-V group in the summer.

During summer season, among the different groups of birds at six week of age, the highest WBC count of $25.67 \pm 1.25 \times 10^3/\mu l$ was observed in G-V (overcrowded birds supplemented with vitamin C) group of birds and the minimum value of $25.17 \pm 1.14 \times 10^3/\mu l$ was observed in G-II (stressed control) group of birds. In the rainy season, WBC count ranged from $25.67 \pm 1.51 \times 10^3/\mu l$ in G-II group of birds to $27.67 \pm 1.61 \times 10^3/\mu l$ in G-I group of birds.

In eight week old birds in rainy season the leukocyte count ranged from $24.83 \pm 1.19 \times 10^3/\mu l$ in G-II group of birds to $26.83 \pm 1.27 \times 10^3/\mu l$ in G-I group of birds. In the second phase, the leukocyte count varied from $26.00 \pm 1.67 \times 10^3/\mu l$ in G-II group of birds to $28.17 \pm 1.60 \times 10^3/\mu l$ in G-I group of birds.

On comparing the TLC value of birds in summer and rainy seasons, irrespective of treatments and ages (4^{th}, 6^{th} and 8^{th}) the difference in TLC between the two seasons (summer as well as rainy seasons) was non significant ($P>0.05$). However, a uniformly low (nonsignificant) TLC value was observed in birds reared under summer when compared to the TLC value of birds reared in the rainy season except in birds in G-V group at eighth week of age.

4.2.6 Differential Leukocyte Count (DLC)

4.2.6.1 Monocyte Count

The effect of overcrowding stress (348cm^2/bird) and effect of some antistress agents viz., Protexin, virginiamycin and vitamin C on the monocyte count of six and eight weeks old birds under overcrowding stress in both summer as well as rainy seasons are presented in table 5.4.

The mean monocyte count of four week old birds in summer varied from 0.75 ± 0.18 per cent in birds of G-IV group to 1.50±0.36 per cent in birds of G-III group. The percentage of monocyte in G-IV and G-V groups of birds (0.75±0.18% and 0.92± 0.23% respectively) were significantly ($P \leq 0.05$) low when compared to those in G-I and G-III group. In the rainy season the initial value of monocyte count in four week old birds ranged from 0.92±0.31 per cent in G-V group of birds to 1.42±0.34 per cent in G-II group of birds. The percentage of monocyte in G-V group of birds was significantly ($P \leq 0.05$) low (0.92±0.31%) when compared to the monocyte count in G-II group of birds. There was no significant ($P > 0.05$) difference in monocyte count of birds in summer and rainy seasons.

After the application of overcrowding stress for a fortnight period, in six week old birds in the summer no significant ($P > 0.05$) change in the monocyte count was observed between the different groups. The count of monocyte in six week old birds in the summer varied from 0.83±0.24 per cent in birds of G-III group to1.25±0.28 per cent in birds of G-I group. In the rainy season among the six week old broiler chicks the highest value of monocyte count was noticed in birds of G-V group (1.50±0.34%). The lowest value of monocyte count was in G-III group of birds (0.92±0.29%). No significant ($P > 0.05$) difference in the monocyte count was noticed between the unstressed control G-I group of birds and stressed control G-II group of birds. The Protexin supplemented G-III group of birds had a monocyte count significantly ($P \leq 0.05$) lower (0.92±0.29%) than birds in other groups. There was no significant (P>0.05) change in the monocyte count of birds at four and six weeks of age.

The monocyte count of eight week old birds in summer ranged from 0.58±0.26 per cent in G-V group of birds to 1.50±0.31 per cent in G-I group of birds. The monocyte count of G-IV and G-V group of birds were significantly (P≤0.05) low when compared to the value in birds of G-I group. In rainy season, the monocyte count of eight week old broilers ranged from 0.75±0.22 per cent in G-I group of birds to 1.25±0.30 per cent in G-III group of birds. The monocyte count in the Protexin fed G-III group of birds was significantly (P≤ 0.05) high (1.25±0.30%) when compared to the control G-I group of birds (0.75±0.22%). No significant (P>0.05) difference in the monocyte count was noticed between the other groups of birds. There was no significant (P>0.05) change in the percentage count of monocyte between six and eight weeks old birds in both summer and rainy seasons. Also no significant difference in the monocyte count existed between eight week old birds reared in summer and rainy seasons.

4.2.6.2 Basophil Count

The results of the study on the effect of stress due to overcrowding and the effect of some antistress feed supplements such as Protexin, virginiamycin and vitamin C on the basophil count of overcrowded birds at six and eight weeks of age in summer and rainy seasons are shown in the table 5.4.

The value of basophil count in four week old broilers (before inducing stress by overcrowding) in summer ranged from 0.35±0.13 per cent in G-III group of birds to 0.47±0.11 per cent in G-II group of birds. No significant (P>0.05) difference in basophil count was noticed between the different groups of birds under study. In the rainy season the basophil count of four week old birds ranged from 0.38±0.23 per cent in birds of G-III group to 0.57±0.22 per cent in birds of

G-V group. Significant variation in the basophil count was observed neither between the different groups of birds nor between birds in summer and rainy seasons.

Among the six week old birds in summer the highest basophil count (0.78±0.22%) was seen in birds of stressed control G-II group and lowest basophil count (0.50±0.15%) was in birds of control G-I group. A significantly ($P \leq 0.05$) high basophil count was observed in G-II group of birds when compared to G-I group of birds. There was no significant ($P > 0.05$) difference in the basophil count noticed between any other groups of birds. The basophil count of birds at fourth and sixth weeks were also statistically similar. In the rainy season the percentage of basophil in six week old birds was maximum (0.85±0.22%) in G-II group and minimum (0.55±0.14%) in G-I group. A significantly ($P \leq 0.05$) high basophil count was noticed in the G-II group of birds when compared to the basophil count in G-I group of birds. No significant ($P > 0.05$) difference in basophil count was evident either between ages (4 and 6 weeks) or between seasons (summer and rainy seasons).

In summer, among the chicks at eighth week of age the basophil count varied from 0.58±0.19 per cent in G-I group of birds to 0.85±0.23 per cent in G-II group of birds. In the rainy season the basophil count in eight week old chicks ranged from 0.52±0.15 per cent in G-IV group of birds to 0.89±0.22 per cent in G-II group of birds.

In both the seasons of the study the basophil count did not differ significantly ($P > 0.05$) between the birds of different ages (4 and 6, 6 and 8 and 4 and 8 weeks) and between the birds of two seasons. However, overcrowding produced increased basophil count in caged broilers at six and eight weeks of age in both summer and rainy seasons.

Table 5.4 Effect of overcrowding stress and antistress agents on haematological parameters (differential leukocyte count) of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameters		Monocyte count (%)			Basophil count (%)			Eosinophil count (%)		
Seasons	Weeks / Groups	4	6	8	4	6	8	4	6	8
S_1	G -I	1.42±0.34$^{ax}_{NS}$	1.25±0.28$^{ax}_{NS}$	1.50±0.31$^{ax}_{NS}$	0.42±0.19$^{ax}_{NS}$	0.50±0.15$^{ax}_{NS}$	0.58±0.19$^{ax}_{NS}$	1.08±0.23^{ax}	0.33±0.14^{ay}	0.33±0.19^{ay}
	G -II	1.00±0.3$^{abx}_{NS}$	1.00±0.28$^{ax}_{NS}$	1.25±0.28$^{abx}_{NS}$	0.47±0.11$^{ax}_{NS}$	0.78±0.22$^{bx}_{NS}$	0.85±0.23$^{bx}_{NS}$	1.08±0.03^{ax}	1.00±0.25^{bx}	0.50±0.19^{ay}
	G -III	1.50±0.36$^{ax}_{NS}$	1.08±0.29$^{ax}_{NS}$	1.33±0.28$^{abx}_{NS}$	0.35±0.13$^{ax}_{NS}$	0.62±0.15$^{ax}_{NS}$	0.60±0.21$^{abx}_{NS}$	0.67±0.28^{bx}	0.75±0.28^{bx}	0.37±0.11^{ax}
	G -IV	0.75±0.18$^{bx}_{NS}$	0.83±0.24$^{ax}_{NS}$	0.92±0.29$^{bcx}_{NS}$	0.38±0.13$^{ax}_{NS}$	0.53±0.21$^{ax}_{NS}$	0.63±0.14$^{abx}_{NS}$	0.53±0.14^{bx} *	0.42±0.19^{ax}	0.33±0.14^{ax}
	G -V	0.92±0.23$^{bx}_{NS}$	1.00±0.30$^{ax}_{NS}$	0.58±0.26$^{cx}_{NS}$	0.37±0.11$^{ax}_{NS}$	0.64±0.22$^{abx}_{NS}$	0.67±0.22$^{abx}_{NS}$	0.42±0.15^{bx}	0.35±0.18^{ax}	0.35±0.18^{ax}
S_2	G -I	1.33±0.31$^{abx}_{NS}$	1.05±0.40$^{abx}_{NS}$	0.75±0.22$^{ax}_{NS}$	0.50±0.23$^{ax}_{NS}$	0.55±0.14$^{ax}_{NS}$	0.62±0.11$^{ax}_{NS}$	1.25±0.30^{ax}	0.48±0.31^{ay}	0.33±0.19^{ay}
	G -II	1.42±0.34$^{ax}_{NS}$	1.42±0.34$^{ax}_{NS}$	1.08±0.31$^{abx}_{NS}$	0.51±0.23$^{ax}_{NS}$	0.85±0.22$^{bx}_{NS}$	0.89 ±0.22$^{bx}_{NS}$	1.00±0. 07^{abx}	0.50±0.23^{ay}	0.47±0.11^{ay}
	G -III	1.00±0.21$^{abx}_{NS}$	0.92±0.29$^{bx}_{NS}$	1.25±0.30$^{bx}_{NS}$	0.38±0.23$^{ax}_{NS}$	0.65±0.18$^{abx}_{NS}$	0.75±0.22$^{abx}_{NS}$	0.92±0.26^{abx}	0.75±0.25^{ax}	0.57±0.26^{ax}
	G -IV	1.33±0.36$^{abx}_{NS}$	1.17±0.24$^{abx}_{NS}$	0.92±0.19$^{abx}_{NS}$	0.47±0.19$^{ax}_{NS}$	0.67±0.19$^{abx}_{NS}$	0.52±0.15$^{ax}_{NS}$	0.92±0.23^{abx} *	0.50±0.15^{ax}	0.58±0.08^{ax}
	G -V	0.92±0.31$^{bx}_{NS}$	1.50±0.34$^{ax}_{NS}$	0.92±0.23$^{abx}_{NS}$	0.57±0.22$^{ax}_{NS}$	0.62±0.19$^{abx}_{NS}$	0.75±0.18$^{abx}_{NS}$	0.82±0.15^{bx}	0.67±0.22^{ax}	0.57±0.11^{ax}

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2), **G -III** Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and **G -V** Overcrowded and vitamin C supplemented group.

S_1 Summer season. **S_2** Rainy season.

a, b, c Means within a column with no common superscripts are significantly different at 5% level

x, y Means within a row with no common superscripts are significantly different at 5% level

* Between seasons significantly different at 5% level

NS No significant difference between seasons s_1 and s_2

4.2.6.3 Eosinophil Count

Table 5.4 represents the eosinophil count of control and experimental groups of birds.

In the summer the percentage of eosinophil in the four week old birds before the application of stress varied from 0.42±0.15 per cent in G-V group of birds to 1.08±0.23 per cent in G-I group of birds. At fourth week of age the eosinophil count in G-III, G-IV and G-V group of birds were significantly ($P \leq 0.05$) low when compared to the G-I and G-II group of birds. In the rainy season, the eosinophil count at fourth week of age ranged from 0.82±0.15 per cent in G-V group of birds to 1.25±0.3 per cent in unstressed control G-I group of birds. A significant ($P \leq 0.05$) difference in the percentage of eosinophil was observed between the G-I and G-V group of birds. Except in birds of G-IV group no significant ($P > 0.05$) difference in eosinophil count was noticed between summer and rainy seasons. In G-IV group of birds the eosinophil count was significantly ($P \leq 0.05$) lower in summer when compared to the birds in rainy season.

Among the six week old birds of summer, a significantly ($P \leq 0.05$) high eosinophil count was noticed in G-II and G-III groups of birds when compared to G-I, G-IV and G-V groups of birds. The percentage of eosinophils varied from 0.33±0.14 per cent in G-I group of birds to 1.00±0.25 per cent in G-II group of birds. In G-I group of birds a significant ($P \leq 0.05$) reduction in percentage of eosinophil was observed from four to six weeks period. In the rainy season the eosinophil count of six week old birds ranged from 0.48±0.31 per cent in birds of G-I group to 0.75±0.25 per cent in birds of G-III group. No significant ($P > 0.05$) variation in the eosinophil count was noticed between different groups of birds. In G-I and G-II group of birds there was a significant ($P \leq 0.05$) reduction in the eosinophil count from four to six weeks period. There was no significant ($P > 0.05$) variation in the eosinophil count between the two seasons.

Among the eight week old birds reared in summer, the highest eosinophil count (0.50±0.19%) was observed in stressed control birds of G-II group and lowest value (0.33±0.14%) was observed in birds of G-IV (overcrowded birds supplemented with virginiamycin) group. In rainy season the eosinophil count was highest (0.58±0.08%) in G-IV group of birds and lowest (0.33±0.19%) in G-I group of birds. There was no significant (P>0.05) difference in the eosinophil count between groups, between ages or between seasons. However, in G-I and G-II groups of birds a significant difference in the eosinophil count was noticed between ages in both seasons.

4.2.6.4 Heterophil Count

The effect of overcrowding stress and antistress agents such as Protexin, virginiamycin and vitamin C on heterophil count of overcrowded birds at fourth, sixth and eighth week of age in summer and rainy seasons are presented in table 5.5.

In the summer season, the heterophil count of four week old birds varied from 30.17±0.61 per cent in G-IV group of birds to 31.92±1.10 per cent in control G-I group of birds. Even before applying stress the percentage of heterophil in G-IV group and G-V group of birds were significantly (P≤0.05) low when compared to the birds in G-I and G-II groups. However, in the rainy season no significant (P>0.05) difference in the initial heterophil count was observed among different groups of birds. The heterophil count of four week old birds in the rainy season ranged from 30.92±0.65 per cent in G-IV group of birds to 32.00±1.08 per cent in G-V group of birds.

In the summer the heterophil count of six week old birds ranged from 38.42±0.43 per cent in control G-I group of birds to 42.08±0.87 per cent in stressed control G-II group of birds. Overcrowding produced a significant (P≤0.05) increase in heterophil count. The antistress feed supplements reduced the heterophil count, which was increased due to overcrowding. Of the three antistress agents used (Protexin, virginiamycin and vitamin C), the vitamin C was found most effective to rectify the increase in heterophil count due to overcrowding. In the rainy season the heterophil count of six week old birds varied from 35.17±1.17 per cent in birds of control G-I group to 39.42±1.42 per cent in birds of Protexin supplemented G-III group. Out of the three antistress agents vitamin C and virginiamycin could decrease the heterophil count increased due to overcrowding. However, contrary to the observations in the summer, in the rainy season Protexin failed to reduce the heterophil count.

There was a significant ($P \leq 0.05$) increase in heterophil count with age indicated by significantly ($P \leq 0.05$) high value of heterophil count in six week old birds when compared to fourth week in both summer as well as rainy seasons. In birds of G-I and G-IV group a significantly high ($P \leq 0.01$) heterophil count observed in the summer than in the rainy season. In all the birds reared in summer the value of heterophil count was higher than their counterparts in the rainy season.

At eighth week of age the heterophil count of birds in the summer ranged from 40.75±0.73 per cent in unstressed control G-I group of birds to 43.75±0.71 per cent in stressed control G-II group of birds. There was a significant ($P \leq 0.05$) increase in percentage of heterophils in stressed control G-II group of birds when compared to other group of birds. Protexin, virginiamycin and vitamin C were all effective in reducing the increased heterophil count resulting from stress due to floor space reduction. In the rainy season the heterophil count of eight week old birds varied from 35.75±1.05 per cent in birds of G-I group to 42.08±1.06 per cent in birds of G-III group. Overcrowding produced increase in heterophil count in eight week old birds in rainy season and among the three antistress feed supplements (Protexin, virginiamycin and vitamin C) only vitamin C could produce a significant ($P \leq 0.05$) reduction to the increase in heterophil count. In all the groups non-significant increase in the heterophil count was noticed between six and eight weeks period. Among the eight week old chicks also the heterophil count was high in the summer than the chicks in the rainy season except in eight week old birds of G-III and G-IV groups. In control G-I and stressed control G-II group of birds the increase was significant (P≤ 0.05).

4.2.6.5 Lymphocyte Count

Table 5.5 displays the data regarding the effect of overcrowding stress and that of antistress agents such as Protexin, virginiamycin and vitamin C on the lymphocyte count of overcrowded birds at fourth, sixth and eighth week of age in summer and rainy seasons.

In the summer season, the lymphocyte count of four week old birds before the application of stress ranged from 65.17±0.83 per cent in G-I group of birds to 68.50±0.60 per cent in G-IV group of birds. The lymphocyte count of G-I group of birds was significantly (P≤0.05) low (65.17±0.83%) when compared to those in G-III, G-IV and G-V groups. The birds in the stressed control G-II group had a significantly (P≤0.05) low lymphocyte count when compared to those in G-IV and G-V groups. However, in the rainy season initially in the four week old birds no significant (P≤0.05) difference in the lymphocyte count noticed between the different groups. The highest lymphocyte count of 66.17±0.82 per cent was observed in G-IV group of birds and lowest lymphocyte count of 65.33±1.46 per cent was noticed in G-I group of birds.

Among the six week old birds in the summer the percentage of lymphocyte was highest (59.50±0.34%) in control G-I group of birds and lowest (55.17±0.73%) in stressed control G-II group of birds. The lymphocyte count of birds in G-II group was significantly (P≤0.05) lower (55.17±0.73%) when compared to the birds in other groups (G-I, G-III, G-IV and G-V). Similar to the results obtained in summer, in the rainy season also, the lymphocyte count of six week old birds in stressed control G-II group was significantly (P≤0.05) the lowest when compared to the other groups (G-I, G-IV and G-V). The lymphocyte count of Protexin supplemented G-III group of birds (58.17±1.19%) was significantly ($P \leq 0.05$) low when compared to the lymphocyte count of birds in G-I, G-IV and G-V groups. The lymphocyte count of six week old birds in rainy season of the experiment ranged from 58.17±1.19 per cent in G-III group of birds to 61.92±1.20 per cent in G-I group of birds.

Among eight week old birds in the summer season the lymphocyte count ranged from 53.92±0.74 per cent in birds of stressed control G-II group to 56.83±0.89 per cent in unstressed control birds of G-I group. The lymphocyte count in stressed control G-II group of birds was significantly ($P \leq 0.05$) low when compared to the other groups (G-I, G-III, G-IV and G-V). Except in the virginiamycin supplemented stressed birds of G-IV group and stressed control birds of G-II group the lymphocyte count decreased significantly ($P \leq 0.05$) from six to eight weeks period. In G-II and G-IV group of birds the reduction in lymphocyte count was not significant ($P > 0.05$). In the rainy season the lymphocyte count of eight week old birds ranged from 55.25±0.96 per cent in Protexin supplemented stressed G-III group of birds to 63.00±1.04 per cent in unstressed control G-I group of birds. The statistical analysis of the data indicated that the lymphocyte count of stressed control birds (G-II group of birds) as well as stressed and supplemented birds (G-III, G-IV and G-V group of birds) were significantly ($P \leq 0.05$) low when compared to the lymphocyte count of unstressed control G-I group of birds. Except in the control G-I group of birds and stressed control G-II group of birds the lymphocyte count of eight week old birds were significantly low ($P \leq 0.05$) when compared to the lymphocyte count of birds at six week of age. In the control group of chicks (G-I and G-II) the lymphocyte count was significantly low ($P \leq 0.05$) in the summer than those chicks in the rainy season. In all the other groups there was no significant ($P > 0.05$) difference in the lymphocyte count observed between the birds in summer and rainy seasons.

Table 5.5 Effect of overcrowding stress and antistress agents on haematological parameters (differential leukocyte count) of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameters		Heterophil count(%)			Lymphocyte count (%)			H/L Ratio		
Seasons	Weeks / Groups	4	6	8	4	6	8	4	6	8
S_1	G -I	31.92±1.10 ^{ax}	38.42±0.43 ^{ay}**	40.75±0.73 ^{az}**	65.17±0.83 ^{ax}	59.50±0.34 ^{ay}	56.83±0.89 ^{az}**	0.49±0.02 ^{ax}	0.65±0.01 ^{ay}*	0.72±0.02 ^{az}**
	G -II	31.75±1.18 ^{ax}	42.08±0.87 ^{by}	43.75±0.71 ^{bz}*	66.00±1.31 ^{abx}	55.17±0.73 ^{by}*	53.92±0.74 ^{by}*	0.48±0.03 ^{ax}	0.77±0.03 ^{by}	0.81±0.03 ^{by}*
	G -III	31.58 ±1.25 ^{abx}	39.67±0.86 ^{ay}	41.58±0.76 ^{az}	67.00±1.10 ^{bcx}	58.08±0.75 ^{acy}	55.92±0.62 ^{az}	0.46±0.03 ^{abx}	0.69±0.03 ^{ay}	0.75±0.02 ^{az}
	G -IV	30.17±0.61 ^{bx}	39.92±0.63 ^{ay}**	41.75±0.80 ^{az}	68.50±0.60 ^{cx}*	58.00±0.55 ^{cy}**	56.67±0.83 ^{ay}	0.44±0.01 ^{bx}	0.69±0.02 ^{ay}**	0.74±0.02 ^{az}
	G -V	30.17±0.98 ^{bx}	38.67±0.83 ^{ay}	41.67±0.5 0 ^{az}*	68.25±1.07 ^{cx}	59.33±0.91 ^{ay}	56.83±0.53 ^{az}	0.44±0.02 ^{bx}	0.65±0.02 ^{ay}	0.73±0.01 ^{az}*
S_2	G -I	31.50±1.00 ^{ax}	35.17±1.17 ^{ay}**	35.75±1.05 ^{ay}**	65.33±1.46 ^{ax}	61.92±1.20 ^{ay}	63.00±1.04 ^{ay}**	0.49±0.03 ^{ax}	0.57±0.03 ^{ay}*	0.57±0.03 ^{ay}**
	G -II	31.17±1.39 ^{ax}	39.08±1.44 ^{by}	41.00±1.08 ^{bcy}*	65.83±1.28 ^{ax}	58.33±1.38 ^{by}*	57.00±1.09 ^{by}*	0.48±0.03 ^{ax}	0.68±0.04 ^{by}	0.72±0.03 ^{bcy}*
	G -III	31.58±0.95 ^{ax}	39.42±1.42 ^{by}	42.08±1.06 ^{bz}	65.92±0.87 ^{ax}	58.17±1.19 ^{by}	55.25±0.96 ^{bz}	0.48±0.02 ^{ax}	0.69±0.04 ^{by}	0.77±0.03 ^{bz}
	G -IV	30.92±0.65 ^{ax}	35.83±0.94 ^{ay}**	41.75±0.73 ^{bcz}	66.17±0.82 ^{ax}*	61.83±1.02 ^{ay}**	56.83±0.55 ^{bz}	0.47±0.02 ^{ax}	0.58±0.02 ^{ay}**	0.74±0.02 ^{bcz}
	G -V	32.00±1.08 ^{ax}	36.08±1.37 ^{ay}	39.83±0.61 ^{cz}*	66.00±1.36 ^{ax}	60.83±1.51 ^{ay}	58.33±0.58 ^{bz}	0.49±0.03 ^{ax}	0.60±0.04 ^{ay}	0.68±0.02 ^{cz}*

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2), **G -III** Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and **G -V** Overcrowded and vitamin C supplemented group.

S_1 Summer season. **S_2** Rainy season.

a, b, c Means within a column with no common superscripts are significantly different at 5% level

x, y, z Means within a row with no common superscripts are significantly different at 5% level

* Between seasons significantly different at 5% level

** Between seasons significantly different at 1% level

H/L Heterophil/ Lymphocyte

4.2.7 Heterophil /Lymphocyte (H/L) Ratio

The H/L ratio of control and treatment groups of birds in both summer and rainy seasons are shown in the table 5.5.

In the summer, the value of H/L ratio in four week old birds before the application of overcrowding stress ranged from 0.44±0.01 in G-IV group of birds to 0.49±0.02 in G-I group of birds. Birds in G-IV and G-V groups had a significantly ($P \leq 0.05$) lower H/L ratio when compared to G-I and G-II groups of birds. In the rainy season, the highest value of H/L ratio was noticed (0.49±0.03) in G-I and G-V groups of birds and lowest (0.47±0.02) in G-IV group of birds. Unlike the difference in H/L ratio observed in summer no significant ($P > 0.05$) difference in H/L ratio was observed in four week old birds of different groups in the rainy season.

The H/L ratio in six week old birds of summer ranged from 0.65±0.01 in G-I group of birds to 0.77±0.03 in G-II group of birds. The stressed control G-II group of birds had a significantly ($P \leq 0.05$) high value of H/L ratio than the unstressed control G-I group of birds. In the summer, all the three feed supplements were effective to reduce the H/L ratio of six week old overcrowded chicks. In the rainy season the H/L ratio varied between 0.57±0.03 in G-I group of birds and 0.69±0.04 in G-III group of birds. As in summer, in the rainy season also, the value of H/L ratio was significantly higher in the stressed control G-II group of birds than the value in the unstressed control G-I group. Of the three feed supplements virginiamycin and vitamin C could reduce the H/L ratio significantly ($P \leq 0.05$). However, Protexin fed G-III group of birds failed to produce a significant ($P \leq 0.05$) reduction in H/L ratio. Birds in all the groups in both seasons had a significant ($P \leq 0.05$) increase in H/L ratio at sixth week when compared to the value at fourth week.

Among the six week old birds in summer and rainy seasons a significant ($P \leq 0.05$) increase in the value of H/L ratio was noticed in G-I group of birds and a highly significant ($P \leq 0.01$) increase in H/L ratio was evident in birds of G-IV group in the summer than those birds reared in the rainy season. In all other group of birds there was no significant ($P > 0.05$) difference in the H/L ratio between summer and rainy seasons.

In eight week old birds also, overcrowding produced a significant ($P \leq 0.05$) increase in value of H/L ratio in both the summer and rainy seasons. In the summer all the three feed supplements were found to be effective in reducing the H/L ratio in overcrowded birds, whereas only vitamin C was found effective in the rainy season. The H/L ratio of eight week old birds in summer varied from 0.72±0.02 in G-I group of birds to 0.81±0.03 in G-II group of birds. Except in the stressed control G-II group of birds a significantly ($P \leq 0.05$) high H/L ratio was observed in eight week old birds when compared to the value in six week old birds.

In the rainy season, among the eight week old birds the highest H/L ratio of 0.77±0.03 was noted in Protexin fed G-III group of birds and lowest value of 0.57±0.03 was noted in unstressed control G-I group of birds. Except vitamin C, other feed additives used could not produce any significant reduction in the H/L ratio. As observed in the summer in the rainy season also the H/L ratio increased significantly ($P \leq 0.05$) with age in the treatment groups of birds. However, no significant increase in H/L ratio was noticed between sixth and eighth weeks of age in G-I and G-II groups of birds. A significantly ($P \leq 0.05$) high H/L ratio was noticed in G-II and G-V groups of birds in the summer season when compared to those in the rainy season. In the unstressed control G-I group of birds the increase was highly significant ($P \leq 0.01$).

4.3 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON BIOCHEMICAL PARAMETERS

4.3.1 Plasma Protein Profile

4.3.1.1 Plasma Total Proteins

Table 6.1 shows the changes in the plasma concentration of total proteins in six and eight weeks old birds subjected to stress by reducing the floor space allowance by 50 per cent when compared to unstressed control birds and birds fed with dietary feed additives such as Protexin, virginiamycin and vitamin C in summer as well as rainy seasons.

In the summer, the concentration of plasma total proteins in four week old birds ranged from 5.03±0.19g/dl in G-III (overcrowded birds supplemented with Protexin) group of birds to 5.15±0.22g/dl in G-II (stressed control) group of birds. In the rainy season the level of plasma total proteins varied from 5.15±0.09g/dl in G-V (overcrowded birds supplemented with vitamin C) group of birds to 5.29±0.08g/dl in G-III group of birds. There was no significant (P>0.05) difference in the levels of plasma total proteins of four week old birds of different groups.

At the sixth week of age, among the overcrowded birds in the summer the stressed control birds of G-II group had the lowest concentration of plasma total proteins 5.11±0.23g/dl. The highest value of 5.38±0.14g/dl was noticed in the control birds of G-I group followed by the vitamin C supplemented stressed birds of G-V group (5.36±0.22g/dl). In the rainy season, the concentration of plasma total proteins in broiler chicken ranged between 5.36±0.12g/dl in G-IV (overcrowded birds supplemented with virginiamycin) group and 5.48±0.08g/dl in G-III (overcrowded birds supplemented with Protexin) group of birds. The overcrowded control birds of G-II group had a low (nonsignificant) protein concentration (5.39±0.12g/dl) when compared to the other groups (G-I, G-III and G-V). Even after the application of overcrowding stress for a fortnight period, no significant (P>0.05) variation was observed in the concentration of plasma total proteins.

Table 6.1 Effect of overcrowding stress and antistress agents on plasma protein profile of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameter		Concentration of total proteins (g/dl)		
Seasons	**Groups \ Weeks**	**4**	**6**	**8**
S_1	G -I	5.13 ±0.18 ^{ax} NS	5.38 ±0.14 ^{ax} NS	5.42 ±0.15 ^{ax} NS
	G -II	5.15 ±0.22 ^{ax} NS	5.11 ±0.23 ^{ax} NS	5.15 ±0.09 ^{ax} NS
	G -III	5.03 ±0.19 ^{ax} NS	5.31±0.12^{axy} NS	5.36 ±0.11 ^{ay} NS
	G -IV	5.09 ±0.13 ^{ax} NS	5.26 ±0.18 ^{ax} NS	5.38 ±0.14 ^{ax} NS
	G -V	5.04 ±0.21 ^{ax} NS	5.36±0.22^{axy} NS	5.43 ±0.16 ^{ay} NS
S_2	G -I	5.19 ±0.07 ^{ax} NS	5.46 ±0.10^{ay} NS	5.45 ±0.10 ^{ay} NS
	G -II	5.18 ±0.09 ^{ax} NS	5.39±0.12 ^{ay} NS	5.41 ±0.09 ^{ay} NS
	G -III	5.29 ±0.08 ^{ax} NS	5.48 ±0.08 ^{ay} NS	5.48 ±0.11 ^{ay} NS
	G -IV	5.17±0.12 ^{ax} NS	5.36±0.12 ^{ay} NS	5.43±0.10 ^{ay} NS
	G -V	5.15±0.09 ^{ax} NS	5.45±0.14 ^{ay} NS	5.47±0.09 ^{ay} NS

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2), **G -III** Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and **G -V** Overcrowded and vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

Means within a column with common superscript did not differ significantly at 5% level

x, y Means within a row with no common superscripts are significantly different at 5% level

NS No significant difference between seasons s_1 and s_2

In the summer eight week old overcrowded birds of G-II group had the lowest (5.15±0.09g/dl) concentration of plasma total proteins and the overcrowded birds supplemented with vitamin C showed the highest (5.43±0.16g/dl) plasma level of total proteins. In the rainy season among the eight week old broilers, the highest value (5.48±0.11g/dl) of plasma total proteins was noticed in G-III group of birds and lowest value of 5.41±0.09g/dl in stressed control G-II group of birds. Application of overcrowding stress did not produce any significant impact on the concentration of plasma total proteins. However, in both summer and rainy seasons, the overcrowding produced a nonsignificant reduction in the concentration of plasma total proteins in six and eight weeks old broiler chicks.

A significant ($P \leq 0.05$) difference in the concentration of plasma total proteins was observed in G-III and G-V groups of birds between fourth and eighth week of age in summer, whereas in the rainy season the concentration of plasma total proteins between four and six weeks as well as four and eight weeks period was significantly ($P \leq 0.05$) different with higher value at six and eight weeks of age compared to that at fourth week.

4.3.1.2 Plasma Albumin

The effect of stress due to floor space reduction (50%) and the influence of some antistress feed supplements on the plasma albumin concentration in six and eight weeks old broiler chicks in summer and rainy seasons are shown in table 6.2.

In both phases of the experiment the albumin level of four week old birds did not differ significantly ($P > 0.05$) between the different groups of broiler chicken. Among the different groups of birds reared in the summer, the highest albumin value of 2.38±0.11g/dl was noticed in G-V (overcrowded birds supplemented with vitamin C) group of birds and the lowest value of 2.28±0.08g/dl was observed in control G-I group of birds. In the rainy season, the albumin level of four week old birds ranged from 2.29±0.05g/dl in G-I group to 2.38±0.07g/dl in G-IV group of birds. No significant ($P > 0.05$) difference in the albumin level was noticed between summer and rainy seasons.

In six week old birds reared in the summer season the concentration of plasma albumin was highest (2.53±0.09g/dl) in G-V (overcrowded birds supplemented with vitamin C) group of birds followed by 2.51±0.05g/dl in G-II group of birds. The lowest value (2.46±0.06g/dl) was seen in G-I (unstressed control) group of birds. In the rainy season, the albumin level of six week old broiler chicken ranged from 2.47±0.06g/dl in control G-I group to 2.84±0.07g/dl in stressed control G-II group of birds. Significantly ($P \leq 0.05$) higher albumin level was noticed in the control birds of G-II group than that of G-I, G-III, G-IV and G-V groups. The virginiamycin supplemented overcrowded birds of G-IV group had the value (2.48±0.06g/dl) very close to that of unstressed control birds of G-I group (2.47±0.06g/dl). On the other hand, the plasma concentration of albumin in G-III and G-V groups of birds were significantly ($P \leq 0.05$) higher than that of G-I group of birds. On comparing the albumin level between the summer and rainy seasons, a highly significant ($P \leq 0.01$) reduction in the albumin level was noticed in six week old birds of G-II group reared in the summer season compared to the G-II group of birds of the rainy season (Table 6.2).

The eight week old broiler chicken in the summer season had a mean albumin level ranging from 2.43±0.14g/dl in G-I (unstressed control) to 2.58±0.09 g/dl in G-II (stressed control) group of birds. There was no significant ($P \leq 0.05$) difference in the albumin concentration of birds of control and treatment groups. Eight week old broilers in the rainy season were found to have an albumin level ranging from 2.52±0.08g/dl in unstressed control birds of G-I group to 2.78±0.04 g/dl in stressed control birds of G-II group. The level of albumin was significantly ($P \leq 0.05$) high in birds of G-II, G-III and G-IV groups when compared to that of control G-I group. The vitamin C supplemented overcrowded broilers of G-V group and stressed and Protexin supplemented broilers of G-III group had an albumin level that was significantly ($P \leq 0.05$) lower than the stressed control birds of G-II group. Virginiamycin supplemented overcrowded birds of G-IV group showed a significantly ($P \leq 0.05$) high albumin concentration (2.68±0.04g/dl) by eighth week of age.

Table 6.2 Effect of overcrowding stress and antistress agents on plasma protein profile of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameters		Concentration of albumin (g/dl)			Concentration of globulin (g/dl)			Albumin: Globulin ratio		
Seasons	Weeks / Groups	4	6	8	4	6	8	4	6	8
S_1	G -I	2.28 ±0.08^{ax}	2.46 ±0.06^{ay}	2.43 ±0.14^{axy}	2.86±0.13^{ax}$_{NS}$	2.91±0.10^{ax}$_{NS}$	2.98±0.16^{ax}$_{NS}$	0.80±0.03^{ax}	0.86±0.02^{ax}	0.82±0.09^{ax}
	G -II	2.32 ±0.12^{ax}	2.51 ±0.05^{ay}**	2.58 ±0.09^{ay}	2.83±0.17^{ax}$_{NS}$	2.59±0.23^{bx}$_{NS}$	2.57±0.09^{bx}$_{NS}$	0.82±0.06^{ax}	0.97±0.18^{ay}	1.00±0.06^{by}
	G -III	2.35 ±0.07^{ax}	2.47 ±0.08^{axy}	2.52 ±0.04^{ay}*	2.67±0.14^{ax}$_{NS}$	2.84±0.14^{ax}$_{NS}$	2.84±0.09^{ax}$_{NS}$	0.88±0.03^{ax}	0.87±0.07^{ax}	0.89±0.03^{abx}
	G -IV	2.38 ±0.07^{ax}	2.46 ±0.09^{axy}	2.51 ±0.07^{ay}*	2.72±0.13^{ax}$_{NS}$	2.82±0.15^{ax}$_{NS}$	2.87±0.11^{ax}$_{NS}$	0.88±0.05^{ax}	0.87±0.06^{ax}	0.87±0.04^{abx}*
	G -V	2.38 ±0.11^{ax}	2.53 ±0.09^{axy}	2.53 ±0.07^{ay}	2.66±0.15^{ax}$_{NS}$	2.84±0.14^{ax}$_{NS}$	2.90±0.11^{ax}$_{NS}$	0.89±0.04^{ax}	0.89±0.03^{ax}	0.87±0.03^{abx}
S_2	G -I	2.29 ±0.05^{ax}	2.47 ±0.06^{ay}	2.52 ±0.08^{ay}	2.90±0.05^{ax}$_{NS}$	2.99±0.08^{ax}$_{NS}$	2.93±0.09^{ax}$_{NS}$	0.79±0.02^{ax}	0.83±0.03^{ax}	0.87±0.05^{ax}
	G -II	2.36 ±0.06^{ax}	2.84 ±0.07^{by}**	2.78 ±0.04^{by}	2.82±0.08^{ax}$_{NS}$	2.55±0.13^{by}$_{NS}$	2.63±0.08^{by}$_{NS}$	0.84±0.04^{ax}	1.11±0.09^{by}	1.06±0.03^{by}
	G -III	2.34 ±0.06^{ax}	2.63 ±0.06^{cy}	2.67 ±0.06^{cy}*	2.94±0.10^{ax}$_{NS}$	2.85±0.09^{ax}$_{NS}$	2.81±0.10^{ax}$_{NS}$	0.80±0.04^{ax}	0.92±0.05^{ay}	0.95±0.05^{aby}
	G -IV	2.38±0.07^{ax}	2.48±0.06^{ax}	2.68±0.04^{bcy}*	2.79±0.08^{ax}$_{NS}$	2.88±0.12^{ax}$_{NS}$	2.76±0.08^{abx}$_{NS}$	0.85±0.03^{ax}	0.86±0.05^{ax}	0.97±0.03^{aby}*
	G -V	2.32±0.04^{ax}	2.62±0.04^{cy}	2.61±0.04^{acy}	2.83±0.07^{ax}$_{NS}$	2.84±0.09^{ax}$_{NS}$	2.86±0.08^{ax}$_{NS}$	0.81±0.02^{ax}	0.92±0.03^{ay}	0.91±0.03^{ay}

G -I Unstressed control (floor space / bird = 696cm^2), G -II Overcrowded control (floor space / bird = 348cm^2), G -III Overcrowded and Protexin supplemented group, G -IV Overcrowded and virginiamycin supplemented group and G -V Overcrowded and vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

a, b, c Means within a column with no common superscripts are significantly different at 5% level

x, y Means within a row with no common superscripts are significantly different at 5% level

* Between seasons significantly different at 5% level

** Between seasons significantly different at 1% level

NS No significant difference between seasons s_1 and s_2

4.3.1.3 Plasma Globulin

The results regarding the effect of overcrowding stress and antistress agents such as Protexin, virginiamycin and vitamin C on the plasma globulin concentration of six and eight weeks old broiler chicks in summer and rainy seasons are depicted in the table 6.2.

The plasma globulin concentration at fourth week of age was not significantly (P>0.05) different among different groups of birds. In the summer the globulin concentration of four week old birds ranged from 2.66±0.15g/dl in G-V (overcrowded birds supplemented with vitamin C) group of birds to 2.86±0.13g/dl in G-I (unstressed control) group of birds. In the rainy season, the level of globulin in four week old birds varied between 2.79±0.08g/dl in G-IV group of birds and 2.94±0.10g/dl in G-III group of birds. No significant (P>0.05) difference in the plasma globulin concentration was observed between the seasons.

At sixth week of age, birds in the summer had the highest globulin level of 2.91±0.10g/dl in G-I (unstressed control) group of birds and lowest level of 2.59±0.23g/dl in G-II (stressed control) group of birds. The concentration of globulin in stressed control G-II group of birds was significantly (P≤0.05) lower when compared to all other groups (G-I, G-III, G-IV and G-V). In the rainy season, the maximum globulin level of 2.99±0.08g/dl was observed in control G-I group of birds and minimum level of 2.55±0.13g/dl was noticed in stressed control G-II group of birds. The birds in G-II group had a significantly (P≤0.05) lower plasma globulin concentration, when compared to G-I, G-III, G-IV and G-V groups of birds. There was no significant (P>0.05) difference in plasma globulin concentration between six week old birds of the two seasons.

In the summer, the plasma globulin concentration in eight week old birds ranged from 2.57±0.09g/dl in G-II group of birds to 2.98±0.16g/dl in G-I group of birds. The stressed control birds of G-II group showed a significantly ($P \leq 0.05$) lower globulin level when compared to the birds of control G-I group, as well as overcrowded birds supplemented with dietary feed supplements (G-III, G-IV and G-V). In the rainy season, the plasma globulin level of eight week old birds ranged from 2.63±0.08g/dl in G-II (stressed control) group of birds to 2.93±0.09g/dl in control G-I group of birds. The stressed control birds of G-II group had a significantly ($P \leq 0.05$) lower globulin concentration when compared to the globulin level of G-I (unstressed control), G-III (Protexin supplemented stressed birds) and G-V (overcrowded birds supplemented with vitamin C) groups of birds. The globulin level (2.76±0.08g/dl) of virginiamycin supplemented stressed birds of G-IV group was between that of G-II group of birds and birds of G-IV and G-V groups. No significant ($P>0.05$) difference was observed in the plasma globulin level of birds in the two seasons.

Even though a significantly ($P \leq 0.05$) lower value of plasma globulin was noticed in six week old broilers of G-II group in the rainy season, seasonal comparison revealed a negative result as far as the variation in the globulin level was concerned. The age of the bird did not produce any significant ($P>0.05$) change in the plasma globulin level.

4.3.2 Albumin Globulin Ratio (A:G Ratio)

Table 6.2 depicts the A:G ratio values of four, six and eight weeks old birds subjected to overcrowding stress and supplemented with dietary antistress agents like probiotic, antibiotic and vitamin C along with the controls (unstressed control G-I and stressed control G-II) in summer and rainy seasons.

Among the four week old birds reared in the summer season, the value of A:G varied from 0.80±0.03 in the control G-I group of birds to 0.89±0.04 in overcrowded birds supplemented with vitamin C (G-V). There was no significant ($P>0.05$) difference in value of A:G between the different groups of birds at fourth week of age immediately before applying the stress (floor space reduction of 50%). In the rainy season, the A:G value of four week old birds ranged from 0.79±0.02 in G-I (unstressed control group) to 0.85±0.03 in G-IV (overcrowded birds supplemented with virginiamycin) group of birds. No significant (P>0.05) difference in value of A:G was noticed between the groups or between the seasons.

In summer, the application of overcrowding (348 cm^2/bird) stress for 14 days increased the A:G value (0.97±0.18) in six week old birds of G-II group. However, the increase was not significant (P>0.05). The highest value (0.97±0.18) of A:G was in G-II and the lowest value (0.86±0.02) was in G-I group of birds. In the rainy season, among the six week old birds the highest value (1.11±0.09) of A:G was observed in birds of G-II (stressed control) group and lowest value (0.83±0.03) was observed in G-I (unstressed control) group of birds. A significantly (P≤0.05) high A:G value was observed in G-II (stressed control) group of birds when compared to the G-I (unstressed control) group of birds and treatment group of birds that received the dietary supplements (Protexin, virginiamycin and vitamin C).

No significant difference in the A:G value was evident in G-I, G-II, G-III, G-IV and G-V groups of birds between summer and rainy seasons. A significantly (P≤0.05) high A:G value was observed between four and six weeks in G-III (overcrowded birds supplemented with Protexin) and G-V (overcrowded birds supplemented with vitamin C) groups of birds in the rainy season and in stressed control G-II group of birds in summer and rainy seasons.

The value of A:G in eight week old broiler chicks in the summer ranged between 0.82±0.09 in birds of G-I (unstressed control) group and 1.00±0.06 in birds of G-II (stressed control) group. Overcrowding stress for 28 days resulted in a significant (P≤0.05) increase in A:G value in eight week old broilers. The feed additives used could not effectively reduce the A:G value in overcrowded (50%

floor space reduction) birds. However, all the feed supplements produced a non-significant reduction in the A:G value resulting in a value, which was not significantly higher than the A:G value of control G-I group of birds. In the rainy season, the A:G value of eight week old birds ranged from 0.87±0.05 in control G-I group to 1.06±0.03 in stressed control G-II group. There was a significant ($P \leq 0.05$) increase in A:G value in stressed control G-II group of birds when compared to G-I group of birds. Of the three antistress supplements only vitamin C had a significant ($P \leq 0.05$) effect in reducing the value of A:G ratio. Protexin as well as virginiamycin were effective in lowering the A:G value, but the reduction was nonsignificant ($P > 0.05$).

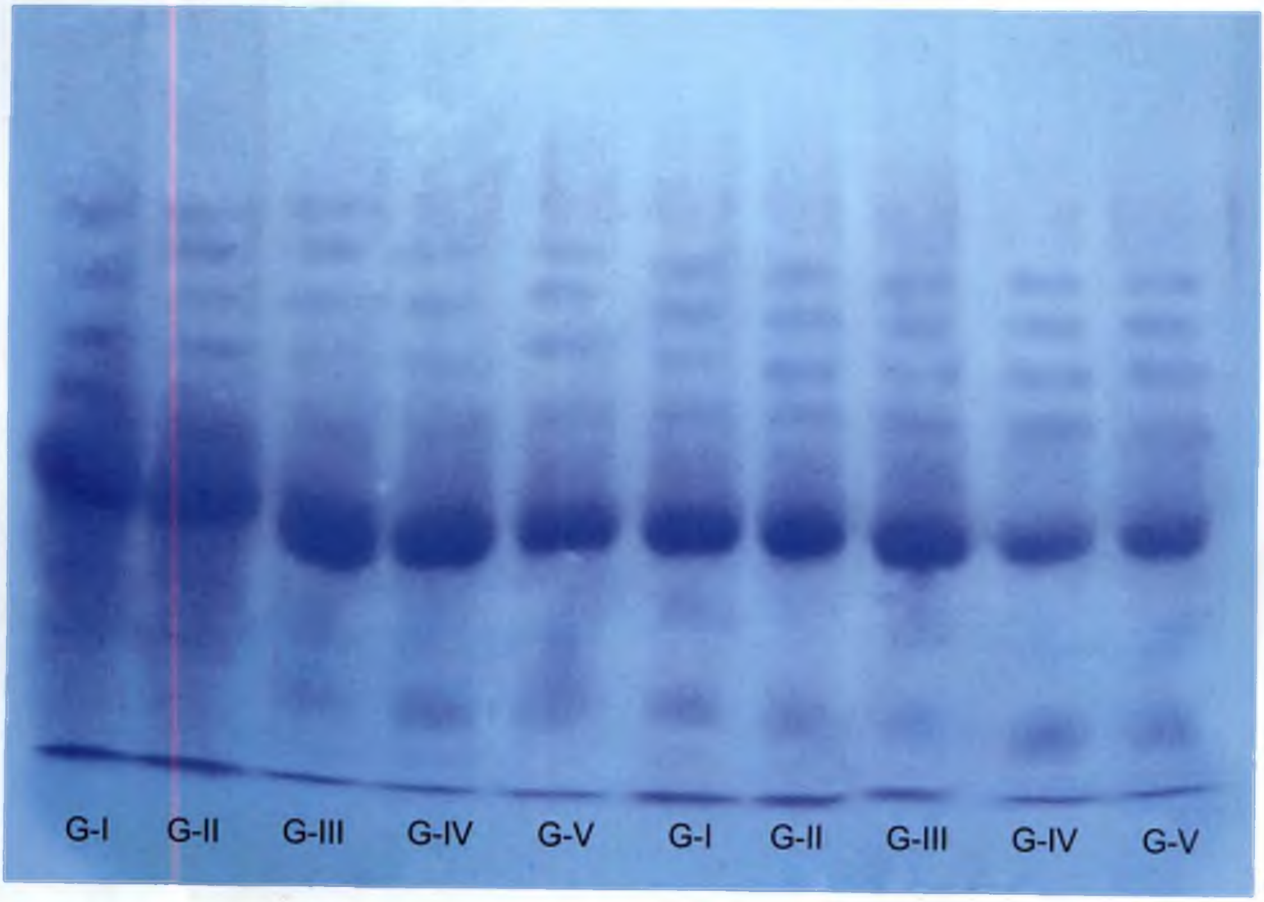
Seasonal comparison of the A:G value showed that no significant ($P > 0.05$) difference in A:G value existed between summer and rainy seasons except in eight week old birds of G-IV group. In the overcrowded virginiamycin supplemented G-IV group of birds the A:G value was significantly lower ($P \leq 0.05$) in the summer when compared to their counter parts in the rainy season.

4.3.3 Electophoretic Seperation of Plasma Total Proteins

The electrophoretogram of the plasma samples from unstressed control (G-I), stressed control (G-II), as well as stressed and antistres agents supplemented groups (G-III, G-IV and G-V) of broiler chicken in summer and rainy seasons are shown in figure 2.

In both seasons the plasma samples produced different Coomassie brilliant blue stained distinct bands up on sodium dodecyl sulphate polyacrylamide gel electrophoresis. A very similar electrophoretic movements were observed in all groups of birds and no extra protein bands were noticed in stressed (G-II) and unstressed (G-II) controls as well as stressed birds supplemented with antistress agents (G-III, G-IV and G-V) in both summer as well as rainy seasons.

Fig. 2 SDS-Polyacrylamide Gel Electrophoretogram of plasma proteins of broiler chicken under the influence of stress and antistress agents in summer and rainy seasons



S_1 S_2

S_1 - Summer S_2 - Rainy Season

4.3.4 Plasma Lipid Profile

4.3.4.1 Total Lipids

The effect of floor space reduction (50%) and that of antistress agents on the plasma total lipid concentration in six and eight weeks old broilers during summer and rainy seasons are given in table 7.

The concentration of plasma total lipids in four week old broiler chicks in summer ranged from 597.02±23.90mg/dl in control G-I group of birds to 628.08±14.63mg/dl in the overcrowded Protexin supplemented G-III group of birds. The birds in all the groups exhibited similar total plasma lipid level. In the rainy season, the level of plasma total lipids varied between 611.51±17.96mg/dl in G-III (overcrowded birds supplemented with Protexin) group of birds and 624.74±18.29mg/dl in G-IV (overcrowded birds supplemented with virginiamycin) group of birds. No significant ($P>0.05$) difference in plasma lipid level was noticed between the different groups. Also no significant ($P>0.05$) difference was observed between summer and rainy seasons.

Among the six week old birds in the summer, the lowest plasma lipid concentration of 625.24±20.96mg/dl was observed in control G-I group of birds and the highest value of 694.23±27.60mg/dl was noticed in stressed control G-II group of birds. Exposure to overcrowding stress for 14 days period resulted in a significantly ($P\leq0.05$) high value of total lipids in the plasma. Protexin and vitamin C could significantly ($P\leq0.05$) reduce the increase in level of plasma total lipids in stressed birds (Table 7). Even though virginiamycin resisted the increase in lipid concentration resulted from overcrowding stress, it failed to produce a significant reduction in the plasma concentration of total lipids. In G-II and G-IV group of birds a significant ($P\leq0.05$) increase in concentration of plasma total lipids noticed between four and six weeks of age (Table 7). In the rainy season, the plasma lipid content of the six week old birds varied between 655.26±10.20mg/dl in G-I (unstressed control) group to 695.95±20.13mg/dl in G-II (stressed control) group. Overcrowded control birds of G-II group showed a significantly ($P \leq 0.05$) high value of plasma total lipids (695.95±20.13mg/dl) when compared to unstressed control G-I group of birds. The level of plasma total lipids of birds in G-III (overcrowded birds supplemented with Protexin), G-IV (overcrowded birds supplemented with virginiamycin) and G-V (overcrowded birds supplemented with vitamin C) groups were between that of unstressed control G-I group and stressed control G-II group. A significantly ($P \leq 0.05$) high value of plasma total lipids was noticed between four and six weeks of age in all the five groups. In G-I, G-II, G-III and G-IV group of chicks there was no significant ($P > 0.05$) difference in the concentration of plasma total lipids between the summer and rainy seasons. However, the concentration of plasma total lipids in birds of G-V (overcrowded birds supplemented with vitamin C) group reared in the summer was significantly ($P \leq 0.05$) less than their counterparts in the rainy season (Table 7). In all the other groups of birds a non-significant reduction in concentration of plasma total lipids was observed in the summer.

During summer season, among the eight week old birds, the stressed control G-II group of birds had the highest (722.89±24.13mg/dl) and overcrowded vitamin C supplemented G-V group of birds had the lowest (647.89±17.19mg/dl) value of plasma total lipids. The overcrowding produced a significantly ($P \leq 0.05$) high plasma total lipids in G-II group of birds when compared to unstressed control G-I group of birds (664.76±13.50mg/dl). In G-I (unstressed control), G-III (overcrowded birds supplemented with Protexin) and G-IV (overcrowded birds supplemented with virginiamycin) groups of birds a significant ($P \leq 0.05$) increase was observed in the plasma lipid concentration between six and eight weeks of age. In all the groups under study, a significant ($P \leq 0.05$) increase in plasma total lipid level was observed between four and eight weeks of age. In rainy season, the eight week old birds of stressed control G-II group had the highest plasma lipid concentration of 715.36±20.13mg/dl and the control G-I group of birds had the lowest plasma lipid concentration of 674.10±11.98mg/dl. The concentration of plasma total lipids was significantly ($P \leq 0.05$) higher (715.36mg/dl) in G-II group of birds when compared to the control G-I group of birds. There was no significant difference noticed between the other groups of birds.

Table 7 Effect of overcrowding stress and antistress agents on plasma lipid profile of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameters		Concentration of Total lipids (mg/dl)			Concentration of Triglycerides (mg/dl)			Concentration of Cholesterol (mg/dl)		
Seasons	Weeks / Groups	4	6	8	4	6	8	4	6	8
S_1	G -I	597.02±23.90^{ax}	625.24±20.96^{ax}	664.76±13.50^{ay}	103.33±3.17^{ax}	109.25±3.11^{ax}	108.53±3.57^{ax}	110.26±2.70$^{ax}_{NS}$	136.94±8.28$^{ay}_{NS}$	158.03±8.93$^{ay}_{NS}$
	G -II	614.12±22.62^{ax}	694.23±27.60^{by}	722.89±24.13^{by}	100.92±2.62^{ax}	113.17±8.31^{ax}	131.97±9.05^{by}	104.23±1.90$^{ax}_{NS}$	174.18±6.95$^{by}_{NS}$	193.27±9.12$^{bz}_{NS}$
	G -III	628.08±14.63^{ax}	632.90±24.96^{ax}	705.60±24.70^{aby}	106.46±3.44^{ax}	105.67±4.32^{ax}	110.92±6.56^{ax}	105.24±2.81$^{ax}_{NS}$	142.09±8.46$^{acy}_{NS}$	163.35±13.40$^{acz}_{NS}$
	G -IV	599.70±24.41^{ax}	652.81±27.69^{aby}	694.90±20.42^{abz}	105.76±6.81^{ax}	107.54±6.41^{ax}	112.59±8.97^{abx}	109.80±2.88$^{ax}_{NS}$	153.17±8.99$^{cy}_{NS}$	174.41±9.01$^{cz}_{NS}$
	G -V	606.64±19.48^{ax}	638.80±16.18^{axy}*	647.89±17.19^{ay}*	110.13±4.68^{ax}	111.40±5.07^{ax}	112.80±6.37^{ax}	111.17±2.71$^{ax}_{NS}$	154.70±7.50$^{cy}_{NS}$	170.33±11.00$^{acy}_{NS}$
S_2	G -I	621.29±13.83^{ax}	655.26±10.20^{ay}	674.10±11.98^{ay}	109.02±6.33^{ax}	114.79±6.42^{ax}	118.14±4.78^{ax}	118.79±8.28$^{ax}_{NS}$	138.03±8.64$^{ay}_{NS}$	157.02±9.26$^{az}_{NS}$
	G -II	617.22±20.99^{ax}	695.95±20.13^{by}	715.36±20.13^{by}	106.10±9.22^{ax}	118.01±6.20^{ay}	121.90±5.23^{ay}	109.48±3.96$^{ax}_{NS}$	172.41±7.11$^{by}_{NS}$	199.62±10.94$^{bz}_{NS}$
	G -III	611.51±17.96^{ax}	679.61±10.72^{aby}	701.10±7.55^{aby}	103.31±4.58^{ax}	115.20±6.22^{ay}	117.13±3.33^{ay}	107.37±4.31$^{ax}_{NS}$	148.30±7.10$^{by}_{NS}$	163.15±10.41$^{acz}_{NS}$
	G -IV	624.74±18.29^{ax}	683.63±13.07^{aby}	708.02±11.85^{abz}	101.71±8.88^{ax}	117.17±6.81^{ay}	123.49±7.21^{ay}	119.56±3.98$^{ax}_{NS}$	161.23±9.75$^{by}_{NS}$	179.62±9.37$^{cz}_{NS}$
	G -V	613.79±13.86^{ax}	680.82±9.67^{aby}*	693.49±8.04^{aby}*	107.05±5.30^{ax}	113.90±4.62^{axy}	119.74±3.86^{ay}	114.71±4.94$^{ax}_{NS}$	163.82±9.01$^{by}_{NS}$	174.77±10.99$^{acy}_{NS}$

G -I Unstressed control (floor space / bird = 696cm^2), G - II Overcrowded control (floor space / bird = 348cm^2), G -III overcrowded and Protexin supplemented group, G -IV Overcrowded and virginiamycin supplemented group and G -V Overcrowded and vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

a, b, c Means within a column with no common superscripts are significantly different at 5% level

x, y, z Means within a row with no common superscripts are significantly different at 5% level

* Between seasons significantly different at 5% level

NS No significant difference between seasons s_1 and s_2

The virginiamycin supplemented stressed birds of G-IV group alone had a significantly ($P \leq 0.05$) high lipid level at eighth week compared to the level at sixth week. However, a significantly ($P \leq 0.05$) increased plasma lipid concentration was noticed in eight week old birds when compared to that in four week old birds. The concentration of total lipids did not vary significantly between the seasons, except in vitamin C supplemented overcrowded birds of G-V group, where a significantly ($P \leq 0.05$) lower value of plasma total lipids was noticed in the summer.

4.3.4.2 Triglycerides

The effect of overcrowding stress and influence of dietary antistress agents on the concentration of triglycerides, in four, six and eight weeks old broiler chicken both in summer and rainy seasons are depicted in the table 7.

The initial value of plasma triglycerides (4^{th} week of age) in birds of different groups was not significantly different. In summer, the plasma triglyceride concentration varied from 100.92±2.62mg/dl in G-II (stressed control) group of birds to 110.13±4.68mg/dl in G-V (overcrowded birds supplemented with vitamin C) group of birds. In the rainy season, the value of plasma triglycerides varied from 101.71±8.88mg/dl in birds of G-IV (overcrowded birds supplemented with virginiamycin) group to 109.02±6.33mg/dl in birds of control G-I group. No significant difference in the plasma triglyceride concentration was noticed between summer and rainy seasons.

In six week old birds during summer, the plasma concentration of triglycerides ranged from 105.67±4.32mg/dl in G-III (overcrowded birds supplemented with Protexin) group of birds to 113.17±8.31mg/dl in G-II (stressed control) group of birds. The variation in the plasma triglyceride concentration was not significant among the different groups. In rainy season, the concentration of plasma triglycerides was highest (118.01±6.20mg/dl) in G-II group of birds and lowest (113.90±4.62mg/dl) in G-V (overcrowded birds supplemented with vitamin C) group of birds. Even though there was no significant change in the plasma triglyceride concentration of six week old birds among the different groups under trial, significantly high ($P \leq 0.05$) level of plasma triglycerides was noticed between four and six weeks old birds in G-II, G-III, and G-IV groups in the rainy season (Table 7). No significant change in the plasma triglyceride value was observed between the two seasons (summer and rainy seasons).

In the summer, among the eight week old birds, the highest value of plasma triglycerides (131.97±9.05mg/dl) was observed in birds of stressed control G-II group and the lowest value (108.53±3.57mg/dl) was observed in unstressed control birds of G-I group. The triglyceride concentration of plasma in stressed control G-II group of birds was significantly ($P \leq 0.05$) higher than the birds of other groups (G-I, G-III and G-V). In the rainy season, among the eight week old chicks, no significant difference was observed in plasma triglyceride concentration between the different groups of birds. The plasma triglyceride level in eight week old birds in rainy season ranged from 117.13±3.33mg/dl in birds of G-III (overcrowded birds supplemented with Protexin) group to 123.49±7.21mg/dl in birds of G-IV (overcrowded birds supplemented with virginiamycin) group. Except in stressed control birds of G-II group no significant change in the plasma triglyceride concentration was noticed between six and eight weeks of age. The triglyceride concentration in plasma between the summer and rainy seasons was also not significantly different among eight week old broiler chicks.

4.3.4.3 Total Cholesterol

Table 7 presents the effect of overcrowding ($348cm^2$/birds) stress and antistress dietary supplements such as Protexin, virginiamycin and vitamin C on the total plasma cholesterol concentration of six and eight weeks old broiler chicks in summer and rainy seasons.

Before applying stress, the plasma cholesterol level in four week old broiler chicks in summer varied from 104.23±1.90mg/dl in G -II (stressed control) group of birds to 111.17±2.71mg/dl in G-V (overcrowded birds supplemented with vitamin C) group of birds. In rainy season, the plasma cholesterol concentration ranged between 107.37±4.31mg/dl in stressed Protexin supplemented (G-III) group of birds and 119.56±3.98mg/dl in stressed and virginiamycin supplemented (G-IV) group of birds. There was no significant difference in the plasma cholesterol concentration either between the different groups or between the two seasons.

Among the six week old birds of summer, the overcrowded control birds of G-II group had the highest cholesterol level of 174.18±6.95mg/dl and unstressed control G-I group of birds had the lowest cholesterol concentration of 136.94±8.28mg/dl. The plasma cholesterol concentration in overcrowded unsupplemented G-II group of birds was significantly ($P \leq 0.05$) higher when compared to all the other groups. Plasma cholesterol concentration of G-IV and G-V group of birds were significantly ($P \leq 0.05$) different from that of control G-I and stressed control G-II group of birds. In the rainy season, the highest cholesterol concentration (172.41±7.11mg/dl) was in G-II (stressed control) and the lowest value (138.03 ±8.64mg/dl) in G-I (unstressed control) group of chicken. The plasma cholesterol concentration in overcrowded unsupplemented G-II group of birds was significantly ($P \leq 0.05$) higher when compared to birds in G-I and G-III group.

Unlike the significant ($P \leq 0.05$) decrease in cholesterol concentration noticed in G-IV (overcrowded birds supplemented with virginiamycin) and G-V (overcrowded birds supplemented with vitamin C) group of birds in the summer, in the rainy season, there was no significant difference in plasma cholesterol content of G-II, G-IV and G-V groups of birds at sixth week. However, the Protexin supplemented overcrowded birds of G-III group showed a significantly ($P \leq 0.05$) low concentration of plasma cholesterol when compared to the stressed control birds of G-II group. No significant difference in the plasma cholesterol concentration was evident between summer and rainy seasons. But a significant ($P \leq 0.05$) increase in the plasma cholesterol concentration was seen between four and six weeks of age in all groups of birds of both seasons.

In summer, among eight week old birds, the plasma cholesterol level varied from 158.03±8.93mg/dl in G-I group of birds to 193.27±9.12mg/dl in G-II group of birds. The concentration of plasma cholesterol in G-II group of birds was significantly ($P \leq 0.05$) higher than the birds of all other groups. Protexin supplemented G-III group of birds and vitamin C supplemented G-V group of birds had a plasma cholesterol content not significantly higher than the normal unstressed birds of the same age. But the virginiamycin supplemented G-IV group of birds showed a plasma cholesterol concentration, which was significantly ($P \leq 0.05$) lower than G-II (stressed control) group of birds and higher than that of control G-I group of birds. In G-II, G-III and G-IV groups of birds the plasma cholesterol level increased significantly ($P \leq 0.05$) from four to eight weeks of age. In the rainy season, the plasma cholesterol concentration of eight week old birds ranged from 157.02 ±9.26mg/dl in control G-I group of birds to 199.62±10.94mg/dl in stressed control G-II group of birds. The birds of G-II group had significantly ($P \leq 0.05$) high plasma cholesterol content when compared to the birds of all other groups. As noticed in the summer in the rainy season too the virginiamycin supplemented overcrowded birds of G-IV group had a cholesterol concentration, which was significantly ($P \leq 0.05$) lower than that of G-II (stressed control) group of birds, but was significantly ($P \leq 0.05$) higher than control G-I group of birds. The cholesterol level of G-III (overcrowded Protexin supplemented birds) and G-V (overcrowded birds supplemented with vitamin C) group of birds showed no significant difference from the G-I (unstressed control) group of birds. During rainy season, except in chicks of G-V group, the increase in the plasma cholesterol concentration between six and eight weeks period was significant ($P \leq 0.05$). In the stressed birds of G-V group, supplemented with vitamin C the increase in cholesterol concentration was only numerical.

The seasonal comparison of plasma cholesterol concentration in four, six and eight weeks old birds indicated no significant difference between summer and rainy seasons on any of the groups.

4.3.5 Plasma Glucose Concentration

Table 8 depicts the plasma glucose concentration in six and eight weeks old broiler chicks under stress due to floor space reduction (50%) in summer and rainy seasons and due to the effects of certain feed supplements such as Protexin, virginiamycin and vitamin C.

In the summer season, the plasma glucose concentration in four week old birds (before the application of stress) varied from 199.03±9.32mg/dl in G-IV (overcrowded birds supplemented with virginiamycin) group of birds to 214.86 ±7.75mg/dl in G-III (overcrowded birds supplemented with Protexin) group of birds. In the rainy season, the value of plasma glucose in four week old chicks ranged between 196.29±4.56mg/dl in G-V (overcrowded birds supplemented with vitamin C) group of birds and 211.96±5.65mg/dl in control G-I group of birds. There was no significant difference in the plasma glucose concentration between the different groups or between the two seasons (summer and rainy).

The plasma glucose level of six week old birds in summer ranged from 239.04±9.09mg/dl in G-IV (overcrowded birds supplemented with virginiamycin) group of birds to 266.71±8.64mg/dl in G-II (stressed control) group of birds. The stressed control birds of G-II group had a plasma glucose value (266.71±8.64mg/dl) significantly ($P \leq 0.05$) higher than all the other groups (G-I, G-III, G-IV and G-V). The plasma glucose level in birds of G-III, G-IV and G-V groups were very close to the plasma glucose level of unstressed control G-I group of birds. In the rainy season the lowest plasma glucose value (228.65±10.89mg/dl) was noticed in virginiamycin supplemented overcrowded G-IV group of birds and the highest value of 257.76±8.52mg/dl in stressed control

G-II group of birds. Similar to the observations in the summer season, in the rainy season also the plasma glucose concentration in stressed control G-II group of birds was significantly (P≤0.05) higher (257.76±8.52mg/dl) than the other groups (G-I, G-III,.G-IV and G-V). A significant (P≤0.05) increase in plasma glucose level was observed between four and six weeks period in all the groups both in summer as well as rainy seasons. There was no significant difference in the plasma glucose concentration in birds reared under summer and rainy seasons.

Among the eight week old birds in summer, the concentration of plasma glucose was highest (271.04±10.23mg/dl) in stressed control G-II group of birds and lowest (248.43±10.09mg/dl) in unstressed control G-I group of birds. The plasma glucose concentration of other group of birds (G-III, G-IV and G-V) was between that of G-I and G-II group of birds. The blood glucose level of birds in G-III and G-IV groups were neither significantly less than that of stressed control G-II group of birds nor significantly higher than that of unstressed control G-I group of birds. There was no significant change in plasma glucose concentration between birds of six and eight weeks except in G-III (overcrowded birds supplemented with Protexin) and G-IV (overcrowded birds supplemented with virginiamycin) group of birds. In G-III and G-IV group of birds the plasma glucose level was significantly (P≤0.05) high at eighth week compared to the value at sixth week. Among the eight week old birds under rainy season, the highest plasma glucose level (272.24±9.07mg/dl) was noticed in G-II (stressed control) group of birds and lowest level (240.23±10.18mg/dl) noticed in G-IV group of birds. The plasma glucose level of G-II group of birds was significantly (P≤ 0.05) higher than that of G-I, G-III, G-IV and G-V group of birds. There was no significant difference in the plasma glucose concentration between six and eight weeks old broilers in any of the groups under investigation. However, a significantly (P≤0.05) higher plasma glucose level was observed between birds of four and eight weeks of age. Seasonal comparison did not reveal any significant change in the plasma glucose concentration between birds reared in summer and rainy seasons.

Table 8 Effect of overcrowding stress and antistress agents on plasma glucose concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameter		Concentration of glucose (mg/dl)		
Seasons	Weeks / Groups	4	6	8
S_1	G -I	200.91±8.46 $^{ax}_{NS}$	239.88±6.75 $^{ay}_{NS}$	248.43±10.09 $^{ay}_{NS}$
	G -II	206.65±9.30 $^{ax}_{NS}$	266.71±8.64 $^{by}_{NS}$	271.04±10.23 $^{by}_{NS}$
	G -III	214.86±7.75 $^{ax}_{NS}$	243.86±8.32 $^{ay}_{NS}$	264.88±9.01 $^{abz}_{NS}$
	G -IV	199.03±9.32 $^{ax}_{NS}$	239.04±9.09 $^{ay}_{NS}$	259.65±11.83 $^{abz}_{NS}$
	G -V	209.53±5.00 $^{ax}_{NS}$	247.72±7.94 $^{ay}_{NS}$	254.37±7.06 $^{ay}_{NS}$
S_2	G -I	211.96±5.65 $^{ax}_{NS}$	233.47±8.57 $^{ay}_{NS}$	241.85±10.09 $^{ay}_{NS}$
	G -II	200.55±3.82 $^{ax}_{NS}$	257.76±8.52 $^{by}_{NS}$	272.24±9.07 $^{by}_{NS}$
	G -III	206.49±3.96 $^{ax}_{NS}$	239.55±7.68 $^{ay}_{NS}$	242.58±11.63 $^{ay}_{NS}$
	G -IV	197.04±4.51 $^{ax}_{NS}$	228.65±10.89 $^{ay}_{NS}$	240.23±10.18 $^{ay}_{NS}$
	G -V	196.29±4.56 $^{ax}_{NS}$	237.76±5.69 $^{ay}_{NS}$	251.22±9.02 $^{ay}_{NS}$

G-I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space/bird = 348cm^2), **G -III** Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and G -V Overcrowded and vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

a, b Means within a column with no common superscripts are significantly different at 5% level

x, y, z Means within a row with no common superscripts are significantly different at 5% level

NS No significant difference between seasons s_1 and s_2

4.4 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON HORMONAL PARAMETERS

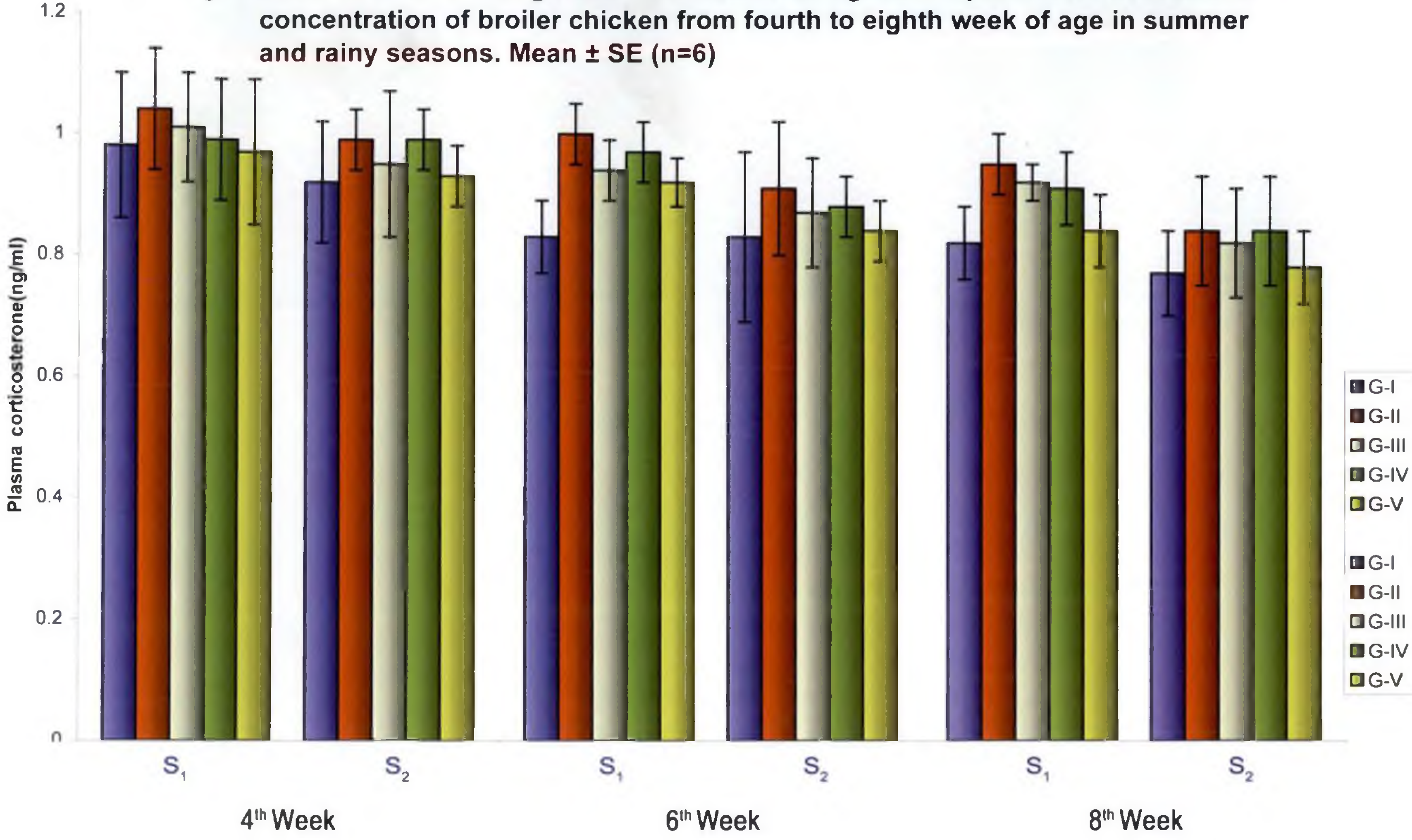
4.4.1 Plasma Corticosterone

The plasma concentration of corticosterone in the control and experimental groups of birds in both summer and rainy seasons are depicted in figure 3.

The corticosterone concentration in four week old birds in summer season ranged from 0.97±0.12ng/ml in G-V group of birds to 1.40±0.10ng/ml in G-II group of birds. In the rainy season the concentration of corticosterone in four week old birds varied from 0.92±0.10ng/ml in unstressed control G-I group of birds to 0.99±0.05ng/ml in stressed control G-II group of birds. There was no significant difference in the concentration of corticosterone either between groups or between seasons.

Among the six week old birds during summer season the corticosterone concentration was maximum (1.00±0.05ng/ml) in G-II group of birds and minimum (0.83±0.06ng/ml) in G-I group of birds. The stressed supplemented group of birds showed the corticosterone value between that of G-I and G-II group of birds. The concentration of corticosterone in G-II group of birds was significantly ($P \leq 0.05$) higher compared to G-I group of birds. The birds in stressed and antistress agents supplemented groups (G-III, G-IV and G-V) had a corticosterone value neither significantly higher than that of G-I group of birds nor significantly lower than that of G-II group of birds. In the rainy season, the concentration of corticosterone in six week old birds varied from 0.83±0.14ng/ml in G-I group of birds to 0.91±0.11ng/ml in G-II group of birds. As in the summer, in the rainy season also, the G-II group of birds had a corticosterone concentration significantly ($P \leq 0.05$) higher than that of control birds of G-I group. Unlike in the summer, in the rainy season supplementation of vitamin C produced a significant ($P \leq 0.05$) reduction in the corticosterone concentration of stressed birds. The birds in G-V group had a corticosterone concentration of 0.84±0.05ng/ml as against 0.91±0.11ng/ml in G-II group. Protexin and virginiamycin produced a non-significant reduction in the corticosterone concentration (Fig. 3).

Fig. 3 Effect of overcrowding stress and antistress agents on plasma corticosterone concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=6)



S_1 - Summer Season **S_2 - Rainy Season**

At the age of eight weeks the birds in summer had a corticosterone concentration ranging from 0.82±0.06ng/ml in G-I group of birds to 0.95±0.05ng/ml in G-II group of birds. There was no significant change in the plasma concentration of corticosterone between different groups of birds. However, the corticosterone concentration in the unstressed control G-I group of birds was lower (nonsignificant) when compared to the other groups. In the rainy season, the value of corticosterone concentration ranged from 0.77 ± 0.07ng/ml in G-I group of birds to 0.84±0.09ng/ml in stressed control G-II group of birds. As observed in the summer season, in the rainy season also the increase in corticosterone concentration in the stressed birds was not significant. The antistress feed supplements also produced no significant effect on the corticosterone concentration of eight week old birds in both summer and rainy seasons.

In summer there was no significant change in the corticosterone concentration between birds of four and six weeks of age. However, a non-significant reduction in the concentration of corticosterone was noticed between birds of four and six weeks of age. During the rainy season, in the unstressed control birds of G-I group and stressed and virginiamycin supplemented birds of G-IV group the value of corticosterone was significantly ($P \leq 0.05$) lower (0.83±0.14ng/ml and 0.88±0.05ng/ml, respectively) at sixth week than the value at fourth week (0.92±0.10ng/ml and 0.99±0.05ng/ml respectively). During summer, no significant difference in the plasma corticosterone level was evident in birds between sixth and eighth week of age. In the G-I group of birds a significantly ($P \leq 0.05$) lower plasma corticosterone level was noticed at eighth week when compared to birds at fourth week of age. During the rainy season also no significant change was observed in the concentration of corticosterone between birds at sixth and eighth week of age. However, a significantly ($P \leq 0.05$) lower corticosterone concentration was observed between four and eight weeks of age in all groups.

Seasonal comparison revealed no significant difference in the plasma corticosterone concentration between the birds during summer and rainy seasons, eventhough a non-significant increase in the value of corticosterone was noticed in birds during summer when compared to rainy season.

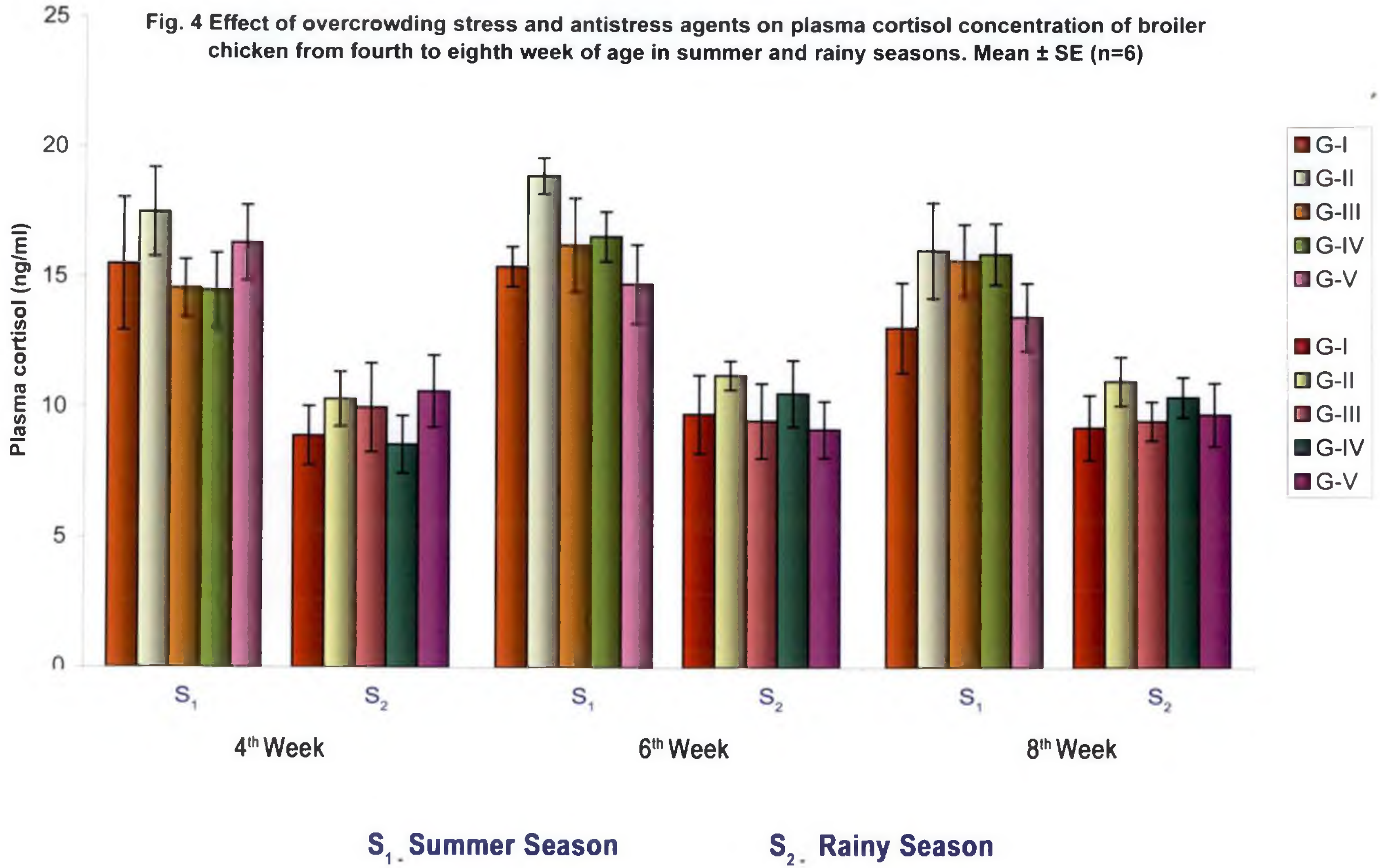
4.4.2 Plasma Cortisol

The cortisol concentration of control and experimental birds in summer and rainy seasons are depicted in figure 4.

The cortisol concentration of four week old birds before application of stress ranged from 14.50±1.44ng/ml in G-IV group of birds to 17.50±1.71ng/ml in G-II group of birds. There was no significant difference in the concentration of cortisol between different groups of birds. In the rainy season the concentration of cortisol varied from 8.58±1.11ng/ml in G-IV group of birds to10.63±1.39ng/ml in G-V group of birds. As in summer, in the rainy season also no significant difference in the cortisol level was noticed between different groups of birds. However, a highly significant ($P \leq 0.01$) difference in the concentration of cortisol was evident between the summer and rainy seasons with a higher value observed in birds during summer season.

Among the six week old birds of summer, the highest (18.92±0.69ng/ml) concentration of cortisol was seen in birds of G-II group and lowest value (14.75±1.52ng/ml) was observed in birds of G-V group. The concentration of cortisol in G-II group of birds was significantly ($P \leq 0.05$) higher when compared to G-I and G-V group of birds. The concentration of cortisol in G-III and G-IV groups of birds were neither significantly higher than G-I and G-V groups of birds nor significantly lower than G-II group of birds. In rainy season among the six week old birds, the highest (11.25±0.56ng/ml) concentration of cortisol was observed in G-II group of birds and the lowest (9.17 ± 1.09ng/ml) value was noticed in G-V group of birds.

Fig. 4 Effect of overcrowding stress and antistress agents on plasma cortisol concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=6)



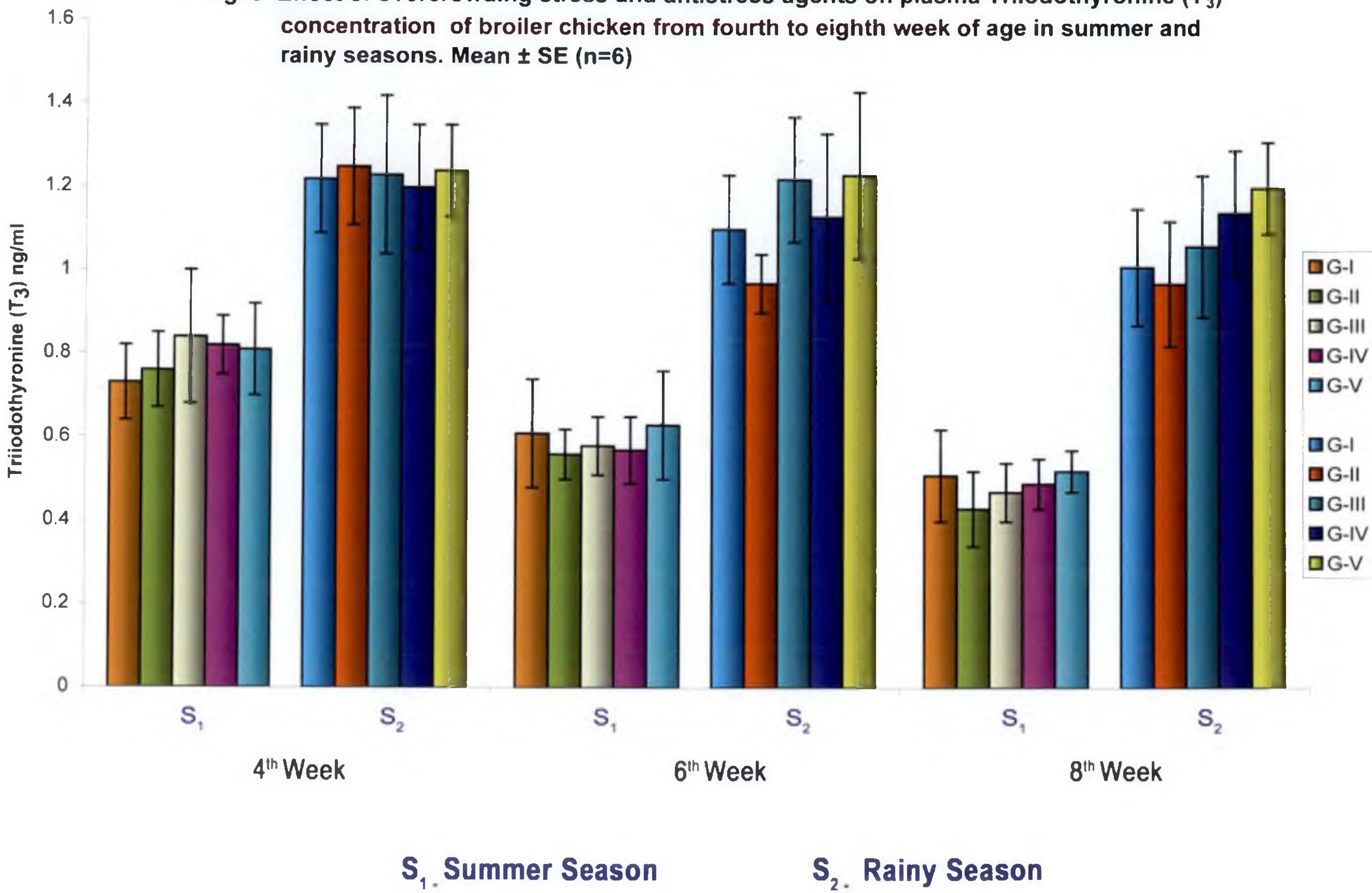
The concentration of cortisol in overcrowded birds of G-II group was significantly ($P \leq 0.05$) higher when compared to the birds in other groups. No significant difference in the cortisol level was noticed between four and six weeks old birds either in summer or in rainy season. Among the six week old birds also, the concentration of cortisol in the summer was significantly higher ($P \leq 0.01$) than their counterparts in the rainy season.

In summer the cortisol level of eight week old birds varied from 13.08±1.73ng/ml in G-I group of birds to 16.06±1.84ng/ml in G-II group of birds. There was no significant change in the concentration of cortisol between the birds in G-II group and birds in the other groups. No significant change in hormone concentration was noticed between six and eight weeks old birds as well as between four and eight weeks old birds. In the rainy season the concentration of cortisol of eight week old birds ranged from 9.25±1.25ng/ml in G-I group to 11.03±0.94ng/ml in G-II group of birds. No significant difference in the concentration of cortisol was noticed between the different groups of birds. As in summer, in the rainy season also no significant change in the cortisol concentration was observed between birds of six and eight weeks as well as between four and eight weeks of age. On seasonal comparison the birds in summer season showed a highly significant ($P \leq 0.01$) increase in the cortisol concentration than in the rainy season. The analysis of the effect of age on the cortisol concentration showed that no significant change in the cortisol concentration existed between birds of four and eight weeks of age in both summer and rainy seasons.

4.4.3 Plasma Triiodothyronine (T_3)

The plasma T_3 concentration in control and experimental groups of birds are expressed in figure 5.

Fig. 5 Effect of overcrowding stress and antistress agents on plasma Triiodothyronine (T_3) concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=6)



In summer, among the four week old birds, the mean plasma concentration of T_3 ranged from 0.73±0.09ng/ml in G-I group of birds to 0.84±0.16ng/ml in G-III group of birds. No significant difference in the plasma T_3 concentration was noticed between the different groups of birds at the beginning of the experiment before application of stress. In the rainy season, the plasma T_3 concentration in four week old birds ranged from1.20±0.15ng/ml in G-IV group of birds to 1.25±0.14ng/ml in G-II group of birds. In the rainy season also, the initial concentration of T_3 before applying the stress did not vary between the birds in different groups.

The concentration of T_3 in six week old birds during summer varied from 0.56±0.06ng/ml in G-II group of birds to 0.63±0.13ng/ml in G-V group of birds. The birds in all the groups had the plasma T_3 concentration not significantly different from each other. However, a significant ($P \leq 0.05$) reduction in the concentration of plasma T_3 was evident from four to six weeks of age in the stressed control as well as with treatment groups of birds. In the control G-I group of birds even though a reduction in plasma T_3 concentration was observed from four to six weeks of age it was not significant. At sixth week of age in the rainy season the highest (1.23±0.20ng/ml) concentration of plasma T_3 was in G-V group of birds and lowest value (0.97± 0.07ng/ml) was in G-II group of birds. Significant ($P \leq 0.05$) difference in the plasma T_3 concentration existed neither between the controls and treatment groups nor between the four and six weeks period. However, in stressed control G-II group of birds a significantly ($P \leq 0.05$) lower plasma T_3 concentration was noticed at sixth week compared to fourth week. In all other groups of birds a non-significant reduction in the plasma concentration of T_3 was observed between four and six weeks period. Among the six week old birds also, the plasma T_3 concentration was significantly ($P \leq 0.05$) lower in summer than in rainy season.

The eight week old birds of summer season showed a plasma T_3 concentration ranging from 0.43±0.09ng/ml in G-II group of birds to

0.52±0.05ng/ml in G-V group of birds. The plasma concentration of T_3 did not vary significantly between the different groups of birds at eighth week of age. No significant difference in T_3 concentration was noticed between the birds at six and eight weeks of age. The concentration of T_3 in birds at eighth week of age was significantly ($P \leq 0.05$) lower compared to fourth week. In the rainy season, the T_3 concentration of eight week old birds ranged from 0.97±0.15ng/ml in G-II group of birds to 1.20±0.11ng/ml in G-V group of birds. Even though overcrowding did not result in any significant change in the plasma T_3 concentration of eight week old broiler chicks, the overcrowded and vitamin C supplemented birds of G-V group had a higher T_3 concentration compared to the birds in stressed control G-II group. In stressed control G-II group the plasma T_3 concentration was significantly ($P \leq 0.05$) lower at eighth week compared to the concentration at fourth week. Unlike the numerical reduction in T_3 concentration noticed at eight week of age than the value at six week of age in all the groups in summer, in the rainy season only G-I, G-III and G-V groups of birds showed a numerical reduction in the concentration of T_3. Among the eight week old birds also, a significantly ($P \leq 0.05$) lower plasma T_3 concentration was noticed in summer than those in the rainy season.

4.4.4 Plasma Thyroxine (T_4)

The mean value of plasma T_4 concentration in four, six and eight weeks old broiler chicks in the control and experimental groups are depicted in the figure 6.

The plasma T_4 concentration in four week old birds (before inducing stress by reducing the floor space allowance/bird) in the summer season varied from 11.33±0.71ng/ml in G-I (unstressed control) group of birds to 12.33±0.61ng/ml in G-III (overcrowded birds supplemented with Protexin) group of birds. There was no significant difference in the concentration of T_4 between the different groups of birds. In the rainy season, the highest concentration of T_4 (16.67±1.52ng/ml) was noticed in the plasma of G-IV (overcrowded birds supplemented with virginiamycin) group of birds and the lowest (14.33±1.05ng/ml) value was noticed in G-II (stressed control) group of birds. The value of plasma T_4 concentration at fourth week of age did not differ significantly between the different groups of birds. However, a significantly ($P \leq 0.05$) high T_4 concentration was observed in all the groups of birds in the rainy season than their counterparts in the summer season.

At the age of six weeks, the lowest concentration of T_4 noticed was 13.17±1.11ng/ml in stressed control G-II group of birds and highest concentration was 14.17±0.54ng/ml observed in unstressed control G-I group of birds. No significant difference in the plasma T_4 concentration was observed between the different groups of birds. Application of overcrowding stress did not produce any significant effect on T_4 concentration in six week old birds in summer. The antistress agents also produced no significant effect on the plasma T_4 concentration of overcrowded birds. However, a significant ($P \leq 0.05$) increase in the plasma T_4 concentration was noticed between four and six weeks of age in all the groups. In the rainy season, the plasma T_4 concentration of six week old birds varied from 15.17±1.47ng/ml in birds of G-V group to 16.83±0.83ng/ml in birds of G-I group. Even though the overcrowded birds had a lower plasma T_4 concentration when compared to the unstressed control G-I group of birds no significant variation in the concentration of plasma T_4 was observed between the birds in control and treatment groups. Among the six week old birds also, the concentration of plasma T_4 in summer was significantly ($P \leq 0.05$) lower than those in the rainy season. Unlike the observations in the summer, in the rainy season there was no significant difference in the plasma T_4 concentration between birds of four and six weeks of age.

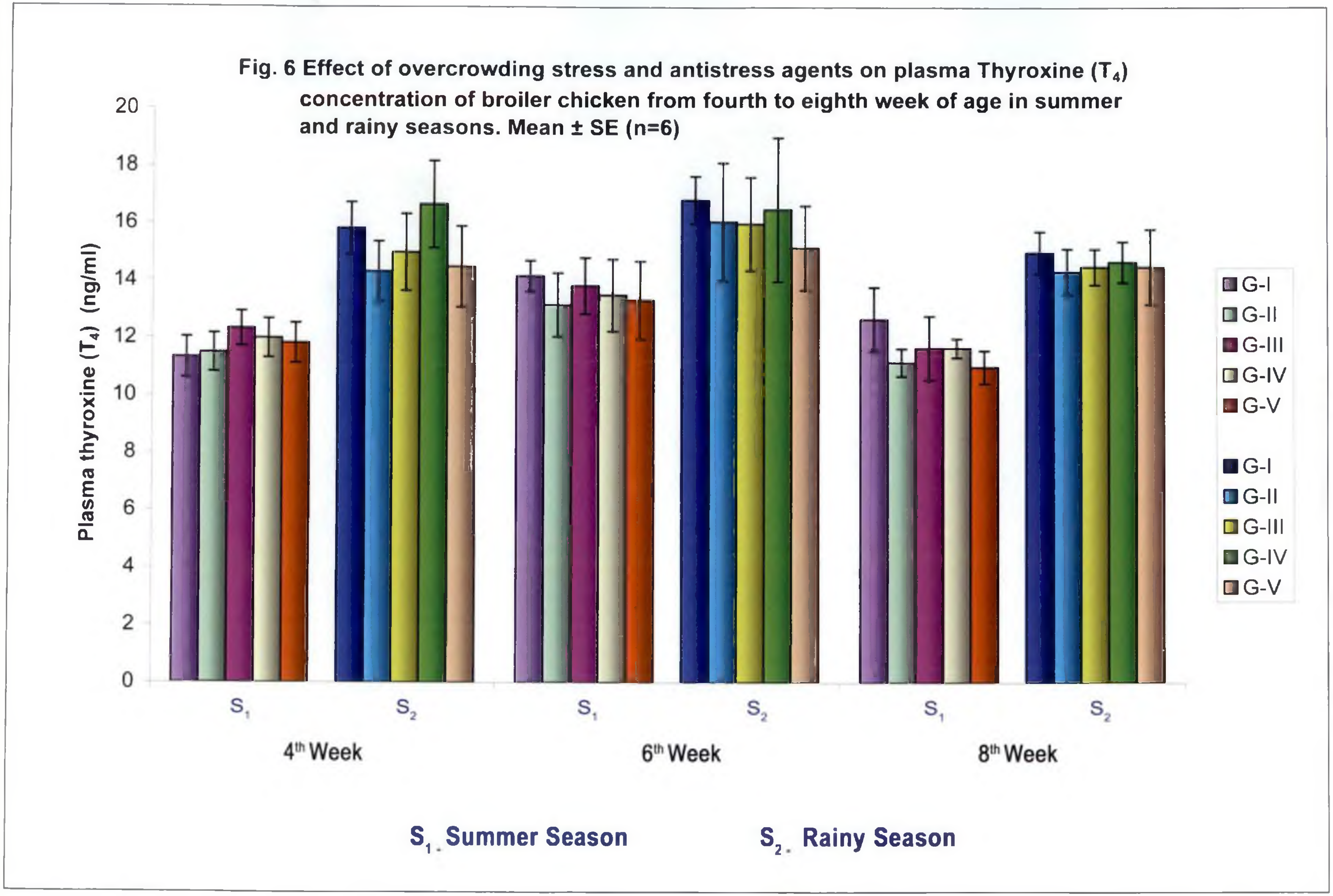


Fig. 6 Effect of overcrowding stress and antistress agents on plasma Thyroxine (T_4) concentration of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=6)

Among the eight week old birds in summer, the plasma T_4 concentration ranged from 11.00±0.58ng/ml in stressed control G-V group of birds to 12.67±1.12ng/ml in unstressed control G-I group of birds. No significant difference in the plasma T_4 concentration was evident between the different groups of birds. The treatment groups of birds had a numerically low plasma T_4 concentration when compared to the control birds of G-I group. The birds at eighth week of age had plasma T_4 value that was significantly ($P \leq 0.05$) lower than the birds at sixth week of age. The plasma T_4 concentration of eight week old birds in all the groups was identical to those at fourth week of age. In the rainy season the plasma T_4 concentration varied from 14.33±0.80ng/ml in stressed control G-II group of birds to 15.00±0.73ng/ml in unstressed control G-I group of birds. There was no significant difference in the plasma T_4 concentration either between, the different groups or between the different ages. However, seasonal comparison showed a significantly ($P \leq 0.05$) lower plasma T_4 concentration in summer than those in the rainy season.

4.5 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON LIVER FUNCTION

4.5.1 Plasma Enzyme Profile

4.5.1.1 Aspartate Amino Transferase (AST) Activity

The effect of overcrowding stress (348cm^2/bird) and antistress agents (Protexin, virginiamycin and vitamin C) on the plasma concentration of AST is shown in table 9.

In the summer, the AST level of four week old birds varied from 159.33±5.69U/l in birds of G-IV (overcrowded birds supplemented with virginiamycin) group to 166.58±4.85U/l in birds of G-V (overcrowded birds supplemented with vitamin C) group. In the rainy season, the AST value ranged between 164.67±4.09U/l in G-I and 172.92±4.41U/l in G-II group of birds. No significant difference in the AST value was noticed between different groups or between the two seasons.

Table 9 Effect of overcrowding stress and antistress agents on plasma enzymes of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=12)

Parameters		AST (U/l)			ALT (U/l)			LDH (U/l)		
Seasons	Weeks / Groups	4	6	8	4	6	8	4	6	8
S_1	G-I	$162.25 \pm 5.76^{ax}_{NS}$	$203.00 \pm 9.96^{ay}_{NS}$	$201.92 \pm 6.08^{ay}_{NS}$	$34.42 \pm 3.91^{ax}_{NS}$	$40.08 \pm 2.75^{aby}_{NS}$	$42.67 \pm 1.98^{ay}_{NS}$	$496.92 \pm 24.27^{ax*}$	758.50 ± 24.31^{ay}	904.75 ± 25.90^{az}
	G-II	$160.25 \pm 5.14^{ax}_{NS}$	$227.08 \pm 12.58^{by}_{NS}$	$259.67 \pm 11.02^{by}_{NS}$	$34.17 \pm 3.49^{ax}_{NS}$	$39.75 \pm 2.77^{abxy}_{NS}$	$44.83 \pm 2.32^{ay}_{NS}$	507.00 ± 26.44^{ax}	898.08 ± 32.05^{by}	964.92 ± 23.66^{bz}
	G-III	$164.75 \pm 5.60^{ax}_{NS}$	$188.25 \pm 9.40^{cy}_{NS}$	$200.00 \pm 12.71^{ay}_{NS}$	$37.17 \pm 2.40^{ax}_{NS}$	$34.83 \pm 2.29^{ax}_{NS}$	$36.67 \pm 1.70^{bx}_{NS}$	497.83 ± 15.55^{ax}	771.67 ± 28.91^{ay}	868.75 ± 26.56^{az}
	G-IV	$159.33 \pm 5.69^{ax}_{NS}$	$201.83 \pm 4.09^{ay}_{NS}$	$205.42 \pm 8.47^{ay}_{NS}$	$38.08 \pm 2.61^{ax}_{NS}$	$40.58 \pm 2.20^{bx}_{NS}$	$42.67 \pm 2.33^{ax}_{NS}$	$511.58 \pm 19.93^{ax*}$	793.33 ± 29.95^{ay}	939.92 ± 21.90^{abz}
	G-V	$166.58 \pm 4.85^{ax}_{NS}$	$193.58 \pm 4.11^{acy}_{NS}$	$211.92 \pm 8.53^{ay}_{NS}$	$34.00 \pm 2.71^{ax}_{NS}$	$35.33 \pm 2.43^{abx}_{NS}$	$37.50 \pm 1.54^{abx}_{NS}$	$483.08 \pm 22.44^{ax*}$	753.83 ± 42.63^{ay}	903.92 ± 25.03^{az}
S_2	G-I	$164.67 \pm 4.09^{ax}_{NS}$	$203.92 \pm 6.37^{ay}_{NS}$	$246.92 \pm 7.35^{az}_{NS}$	$34.25 \pm 1.87^{ax}_{NS}$	$39.50 \pm 1.78^{ay}_{NS}$	$40.00 \pm 1.95^{aby}_{NS}$	$565.75 \pm 22.77^{ax*}$	691.00 ± 45.22^{ay}	815.92 ± 45.93^{az}
	G-II	$172.92 \pm 4.41^{ax}_{NS}$	$225.33 \pm 8.91^{by}_{NS}$	$251.75 \pm 6.78^{az}_{NS}$	$33.67 \pm 2.43^{ax}_{NS}$	$41.08 \pm 3.37^{ay}_{NS}$	$44.92 \pm 2.57^{bz}_{NS}$	568.67 ± 25.91^{ax}	812.67 ± 38.01^{by}	943.50 ± 38.30^{bz}
	G-III	$167.33 \pm 5.94^{ax}_{NS}$	$205.25 \pm 4.39^{ay}_{NS}$	$219.00 \pm 5.21^{bz}_{NS}$	$34.33 \pm 2.83^{ax}_{NS}$	$36.58 \pm 2.59^{ax}_{NS}$	$37.50 \pm 2.50^{ax}_{NS}$	530.92 ± 37.31^{ax}	727.17 ± 41.30^{ay}	835.83 ± 34.57^{az}
	G-IV	$166.33 \pm 3.96^{ax}_{NS}$	$196.50 \pm 3.46^{acy}_{NS}$	$207.75 \pm 5.79^{bz}_{NS}$	$33.25 \pm 2.78^{ax}_{NS}$	$38.08 \pm 2.45^{ay}_{NS}$	$41.08 \pm 2.84^{aby}_{NS}$	$575.00 \pm 23.23^{ax*}$	761.50 ± 34.47^{aby}	879.25 ± 24.72^{abz}
	G-V	$166.83 \pm 3.41^{ax}_{NS}$	$186.50 \pm 3.22^{cy}_{NS}$	$211.50 \pm 5.63^{bz}_{NS}$	$33.00 \pm 3.12^{ax}_{NS}$	$35.42 \pm 2.79^{axy}_{NS}$	$36.75 \pm 2.31^{ay}_{NS}$	$573.58 \pm 27.54^{ax*}$	754.50 ± 28.97^{aby}	838.17 ± 36.36^{az}

G -I Unstressed control (floor space / bird = $696cm^2$), **G -II** Overcrowded control (floor space / bird = $348cm^2$), **G -III** Overcrowded Protexin supplemented group,

G -IV Overcrowded virginiamycin supplemented group and **G -V** Overcrowded vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

a, b, c Means within a column with no common superscripts are significantly different at 5% level

x, y, z Means within a row with no common superscripts are significantly different at 5% level

* Between seasons significantly different at 5% level

NS No significant difference between seasons s_1 and s_2

AST Aspartate amino transferase **ALT** Alanine amino transferase **LDH** Lactate dehydrogenase

In summer, the six week old birds in G-III, G-IV and G-V groups showed a significantly ($P \leq 0.05$) lower AST value when compared to stressed control (G-II) group of birds. The AST level observed in the control (G-I) birds was between that of G-II and the other groups. The highest AST value (227.08±12.58U/l) was seen in G-II group of birds and the lowest level (188.25±9.40U/l) was observed in G-III (overcrowded bird supplemented with Protexin) group of birds.

In the rainy season, the activity of AST in six week old broiler chicks ranged from 186.50±3.22 U/l in G-V to 225.33±8.91 U/l in G-II group of birds. A significantly ($P \leq 0.05$) high AST activity was noticed in overcrowded birds, of G-II group. All the feed additives used effectively reduced the AST level in overcrowded (29 birds/m^2) birds.

The AST value of eight week old birds of summer ranged from 200.00±12.71 U/l in G-III group of birds to 259.67±11.02 U/l in G-II group of birds. A significantly ($P \leq 0.05$) increased AST level (259.67±11.02U/l) was observed in overcrowded control group of birds in the summer. No significant difference in AST activity was observed between the unstressed control (G-I) group of birds or overcrowded birds fed with feed additives.

In the rainy season, it was seen that the enzyme level of eight week old broiler birds were not affected by the floor space reduction (50%) applied. A significantly ($P \leq 0.05$) low AST level was observed in stressed birds receiving antistress agents (G-III, G-IV and G-V), when compared to the unstressed control G-I and stressed control G-II group of birds. The value of AST ranged from 207.75±5.79U/l in birds of G-IV (overcrowded birds supplemented with virginiamycin) group to 251.75±6.78U/l in birds of G-II (stressed control) group.

The AST value showed an increasing trend from four to six weeks of age. On comparing the AST value between the two seasons, no significant difference in the value of AST was noticed between summer and rainy seasons, at fourth, sixth or eighth week of age in any of the groups.

4.5.1.2 Alanine Amino Transferase (ALT) Activity

Results regarding the effect of overcrowding stress (348 cm^2/bird) and antistress dietary supplements (Protexin, virginiamycin and vitamin C) on ALT activity of six and eight weeks old broiler chicken is presented in table 9.

The value of ALT at fourth week of age before inducing the overcrowding stress was the highest (38.08±2.61U/l) in G-IV (overcrowded birds supplemented with virginiamycin) group of birds and lowest (34.00±2.71U/l) in G-V group of birds during summer. In the rainy season, the ALT level varied from 33.00±3.12U/l in G-V group to 34.33±2.83U/l in G-III (overcrowded birds supplemented with Protexin) group.

In both seasons, after applying the overcrowding stress (50% reduction in floor space) for a period of 14 days, the birds at sixth week of age did not show any significant change in the plasma ALT activity. The ALT activity in six week old birds in the summer ranged from 34.83±2.29U/l in G-III group of birds (overcrowded birds supplemented with Protexin) to 40.58±2.20U/l in G-IV (overcrowded birds supplemented with virginiamycin) group of birds. The ALT activity of G-III group of birds was significantly lower ($P \leq 0.05$) than G-IV group of birds. In rainy season, the ALT activity varied from 35.42±2.79U/l in G-V group of birds to 41.08±3.37U/l in G-II (stressed control) group of birds. No significant change in ALT activity was observed between birds reared in summer and rainy seasons. The overcrowded birds supplemented with vitamin C (G-V group) in summer also had a lower ALT activity (35.33±2.43U/l). On the other hand in the rainy season, no significant change was observed between the ALT activity of control and treatment groups.

On comparing the ALT level of eight week old birds in the summer, it was seen that the ALT level of G-III group of birds was significantly (P≤0.05) lower when compared to the ALT level of G-I, G-II and G-IV group of birds. The level of ALT in G-V group of birds was in between that of G-III and the other groups (G-I, G-II and G-IV). However, overcrowding (50% reduction in floor space) did not produce any significant effect on the ALT level of eight week old birds in both seasons (Table 9).

In summer, the ALT level of eight week old birds varied from 36.67±1.70U/l in G-III group of birds (overcrowded birds supplemented with Protexin) to 44.83±2.32U/l in G-II (stressed control) group of birds. In the rainy season, the ALT level ranged from 36.75±2.31U/l in overcrowded birds supplemented with vitamin C (G-V) to 44.92±2.57U/l in G-II (stressed control) group of birds. Seasonal variation in ALT level in various groups studied was not significant at eighth week of age.

In the rainy season, the probiotic supplemented overcrowded birds of G-III group and Vitamin C supplemented overcrowded birds of G-V group had a lower ALT level when compared to the other groups (G-I, G-II and G-IV). A significant (P≤0.05) reduction in the ALT level was observed in birds of G-III (37.50±2.50U/l) and G-V (36.75±2.31U/l) groups when compared to the ALT level of stressed control birds of G-II (44.92±2.57U/l) group.

Fluctuating tendency in the ALT activity was evident in both controls and treatment group of birds during the entire trial period from four to eight weeks period. No significant variation in ALT activity was observed between the summer and rainy seasons.

4.5.1.3 Lactate Dehydrogenase (LDH) Activity

The effect of overcrowding stress (50% reduction in floor space) and antistress agents (Protexin, virginiamycin and vitamin C) on plasma LDH level of six and eight weeks old broilers in summer and rainy seasons are depicted in table 9.

At fourth week of age, plasma LDH activity was identical in all groups of birds. The value at fourth week of age for the activity of LDH among different groups of chicken varied from 483.08±22.44U/l in G-V (overcrowded birds supplemented with vitamin C) group to 511.58±19.93U/l in G-IV (overcrowded birds supplemented with virginiamycin) group. During the rainy season the LDH activity in four week old birds ranged from 530.92±37.31U/l in G-III group of birds to 575.00±23.23U/l in G-IV group of birds. In G-I, G-IV and G-V groups the LDH level was significantly (P≤0.05) lower in the summer than those in the rainy season.

In summer and rainy seasons, the application of overcrowding stress for 14 days produced a significant (P≤0.05) increase in the LDH activity in birds at sixth week of age. In summer, the value of LDH was significantly (P≤0.05) lower in all the treatment groups (G-III, G-IV and G-V), when compared to the LDH level of overcrowded control birds of G-II group (898.08±32.05U/l). The LDH level of six week old birds in the summer ranged from 753.83±42.63U/l in birds of G-V group (overcrowded birds supplemented with vitamin C) to 898.08±32.05U/l in birds of G-II (stressed control) group. The LDH level of vitamin C supplemented overcrowded birds (753.83±42.63U/l) were even lower (numerically) than the unstressed control birds (758.50±24.31U/l). In the rainy season, the LDH level of six week old birds ranged from 691.00±45.22U/l in control G-I group of birds to 812.67±38.01U/l in G-II group of birds (stressed control). The LDH level was significantly (P≤0.05) lower in G-I (unstressed control) and G-III (overcrowded birds supplemented with Protexin) group of birds, when compared to the LDH level of G-II (stressed control) group of birds. The level of LDH in G-IV (overcrowded birds supplemented with virginiamycin) and G-V (overcrowded birds supplemented with vitamin C) group of birds were between the values of G-I and G-II groups. No significant reduction in the LDH level was noticed between G-II and G-V groups of birds of rainy season.

The LDH activity of eight week old birds varied between 868.75±26.56U/l in birds of G-III group (overcrowded birds supplemented with Protexin) and 964.92±23.66U/l in birds of G-II group (stressed control). The LDH level (964.92±23.66U/l) was significantly ($P \leq 0.05$) higher in overcrowded control (G-II) birds. The probiotic Protexin as well as vitamin C produced a significant ($P \leq 0.05$) reduction in the LDH level of overcrowded birds at eighth week of age. The value of LDH in stressed, virginiamycin supplemented G-IV group of birds (939.92±21.90U/l) was also lower (nonsignificant) than that of (964.92±23.66U/l) stressed control (G-II) group of birds.

In the rainy season, the LDH level of eight week old broilers ranged between 815.92±45.93U/l in G-I (unstressed control) group and 943.50±38.30U/l in G-II (stressed control) group. The level of LDH of broilers in G-II group was significantly ($P \leq 0.05$) higher (943.50±38.30U/l) compared to G-I, G-III and G-V groups of birds. In the G-IV group of birds the value of LDH activity was numerically lower (879.25± 24.72U/l) than that of G-II group (943.50±38.30U/l) of birds.

In both summer as well as rainy seasons it was seen that the LDH activity increased significantly with age (4 to 8 weeks). Seasonal comparison of the data revealed that the LDH level of G-I, G-IV and G-V group of birds at fourth week of age was significantly ($P \leq 0.05$) lower in the summer compared to their counter parts in the rainy season. No significant change in the LDH activity was noticed between summer and rainy seasons in six and eight weeks old birds.

In both control and treatment groups of birds of summer and rainy seasons a significant ($P \leq 0.05$) increase in the value of LDH was evident with increase in age of the bird.

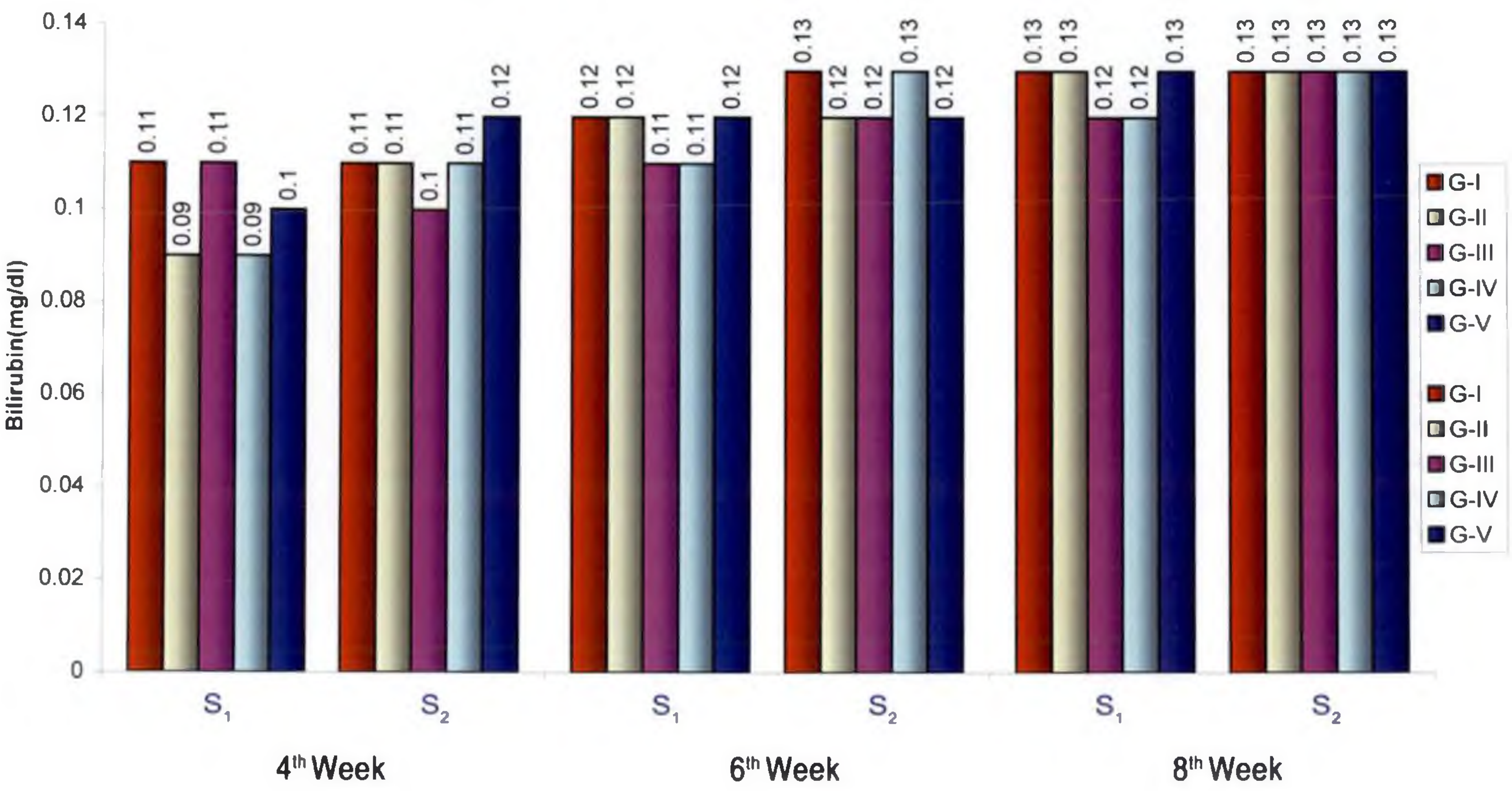
4.5.1.4 Plasma Bilirubin Concentration

The plasma bilirubin concentration of control and treatment groups of birds are depicted in figure 7.

The concentration of bilirubin in four week old birds in summer ranged from 0.09mg/dl in G-II and G-IV group of birds to 0.11mg/dl in G-I and G-III groups of birds. In the rainy season the concentration of bilirubin ranged from 0.10mg/dl in G-III group of birds to 0.12mg/dl in G-V group of birds. Much variation was not observed in the concentration of bilirubin between the different groups or between the two seasons.

Among the six week old birds the highest value (0.12mg/dl) of plasma bilirubin concentration was noticed in G-I, G-II and G-V groups of birds. The lowest (0.11mg/dl) value of bilirubin concentration was observed in birds of G-III and G-IV groups. In the rainy season the bilirubin concentration of six week old birds varied from 0.12mg/dl in G-II, G-III and G-V groups of birds to 0.13mg/dl in G-I and G-IV groups of birds. At sixth week also not much variation in the bilirubin concentration was observed either between groups or between seasons. Application of overcrowding stress for a fortnight period did not produce any effect on the bilirubin concentration of plasma. Combined effect of heat and overcrowding also proved to have no effect on the plasma bilirubin concentration in broiler chicks.

Fig. 7 Effect of overcrowding stress and antistress agents on plasma bilirubin concentration in broiler chicken from fourth to eighth week of age in summer and rainy seasons. (n=12)



S_1 . Summer Season **S_2 . Rainy Season**

At the age of eight weeks, the birds reared in the summer had a bilirubin concentration varying from 0.12mg/dl in G-III and G-IV groups to 0.13mg/dl in G-I, G-II and G-V groups. In the rainy season the chicks in all the groups showed the same mean bilirubin value of 0.13mg/dl. The seasonal variation in bilirubin concentration was also meagre.

4.6 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON THE IMMUNITY OF BROILER CHICKEN

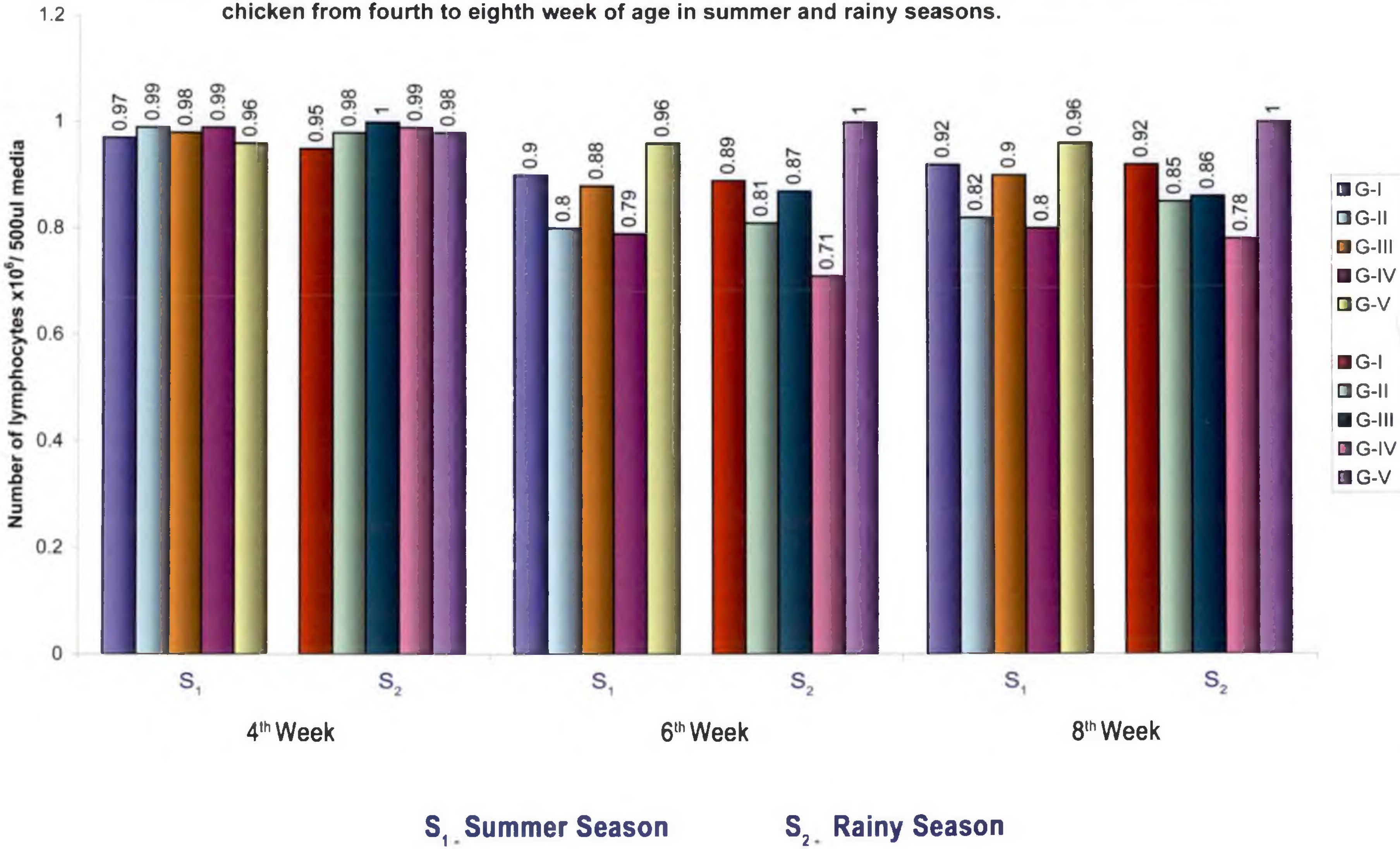
4.6.1 Lymphoblastogenic Response

The effect of overcrowding stress and antistress feed supplements on the mitogen - induced lymphoblastogenic response of four, six and eight weeks old broiler chicken in summer and rainy seasons are given in figure 8.

After an incubation period of 72h, the isolated lymphocytes responded to plasma from four week old birds (before applying stress) to the extend of 0.99×10^6cells per 500µl in G-II and G-IV groups of birds to 0.96×10^6cells per 500µl of medium in G-V group of birds, from a base value of 0.5×10^6cells/500 µl. In the rainy season, the highest value (1.00×10^6cells/500µl) was seen in G-III group of birds and the lowest value (0.95×10^6cells /500µl) was noticed in control G-I group of birds.

At the age of six week the mitogen induced lymphocyte blastogenic response was less in virginiamycin supplemented overcrowded G-IV group of birds (0.79×10^6cells/500µl) and overcrowded unsupplemented G-II group of birds (0.80×10^6cells/500µl). The birds in vitamin C supplemented overcrowded group (G-V) produced a lymphocyte proliferative response (0.96×10^6cells/500µl), which was even higher than that of unstressed control G-I group of birds (0.90×10^6cells/500µl).

Fig. 8 Effect of overcrowding stress and antistress agents on lymphoproliferative response in broiler chicken from fourth to eighth week of age in summer and rainy seasons.



In the rainy season, the lymphoblastogenic response of six week old broiler chicks ranged from $0.71x10^6$cells/500μl in G-IV (overcrowded birds supplemented with virginiamycin) group of birds to $1.00x10^6$cells/500μl in G-V (overcrowded birds supplemented with vitamin C) group of birds. The birds of G-II, G-III and G-IV groups had a lymphoblastogenic response lesser than that of control birds in both summer and rainy seasons (Fig.8).

Among the eight week old birds during the summer season, the highest value of lymphoblastogenic response ($0.96x10^6$cells/500μl) was observed in birds of G-V (overcrowded birds supplemented with vitamin C) group and the lowest value ($0.80x10^6$cells/500μl) was observed in birds of G-IV (overcrowded birds supplemented with virginiamycin) group. The stressed control birds of G-II group showed a lymphocyte proliferation value of $0.82x10^6$cells/500μl.

In the rainy season the lymphocyte proliferation response of eight week old broiler chicken varied from $0.78x10^6$cells/500μl in G-IV (overcrowded birds supplemented with virginiamycin) group of birds to $1.00x10^6$cells/500μl in G-V (overcrowded birds supplemented with vitamin C) group of birds. The stressed control birds of G-II group showed a lymphoblastogenic response value of $0.85x10^6$ cells/500μl.

4.6.2 Concentration of Immunoglobulin G (IgG)

The results regarding the effect of overcrowding stress and antistress agents on the IgG concentration determined by the single radial immunodiffusion (Fig.9) of six and eight weeks old birds in summer and rainy seasons are depicted in figure 10.

In the summer, the plasma IgG level of four week old birds varied from 2mg/ml in G-V group of birds to 3.6mg/ml in G-II group of birds. In the rainy season the IgG concentration of four week old birds ranged from 3.15mg/ml in G-V group of birds to 3.80mg/ml in G-I, G-III and G-IV groups of birds.

Fig. 9 Radial immuno - diffusion method for quantification of immunoglobulin G (IgG)

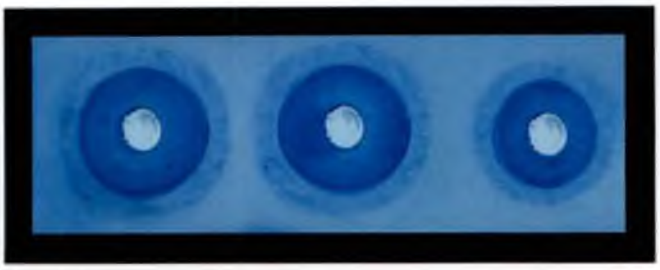
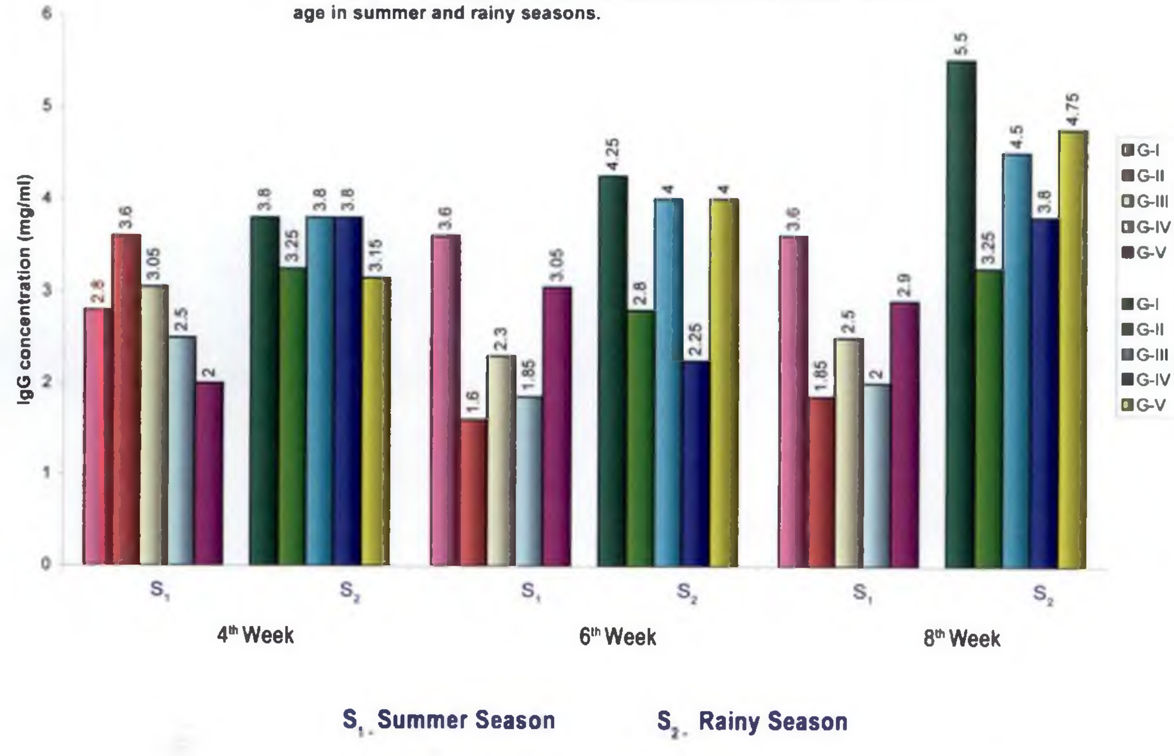


Fig. 10 Effect of overcrowding stress and antistress agents on concentration of immunoglobulin G (IgG) in broiler chicken from fourth to eighth week of age in summer and rainy seasons.



Among the six week old birds in the summer season the plasma IgG value varied from 1.60mg/ml in stressed control G-II group of birds to 3.60mg/ml in unstressed control G-I group of birds. The birds in G-IV (overcrowded birds supplemented with virginiamycin) group also showed a lower value (1.85mg/ml) of IgG. The G-III (overcrowded birds supplemented with Protexin) group of birds had a value of IgG (2.30mg/ml) less than that of G-I (3.60mg/ml) and G-V (3.05mg/ml) group of birds. The six week old birds in the rainy season showed an IgG concentration ranging from 2.25mg/ml in G-IV group of birds to 4.25mg/ml in G-I group of birds. The birds in G-II group showed a mean IgG value of 2.80mg/ml, which was also lower than that of G-I group of birds.

The IgG concentration in eight week old birds in the summer varied from 1.85mg/ml in G-II group of birds to 3.60mg/ml in G-I group of birds. The G-IV (overcrowded birds supplemented with virginiamycin) group of birds also showed a lower concentration of IgG when compared to the unstressed control G-I group of birds. Among the eight week old birds in the rainy season the IgG level was maximum (5.50mg/ml) in G-I group of birds and minimum (3.25mg/ml) in G-II group of birds. The G-IV group of birds also showed a reduced value of IgG (3.80mg/ml). In the unstressed control G-I group of birds the value of IgG increased with increase in age, whereas in all other groups the IgG value fluctuate between four and eight weeks period.

In summer the plasma IgG value increased with age only in unstressed control G-I group of birds. However, during the rainy season, in G-I, G-III and G-V groups of birds, an increase in the concentration of IgG was observed from four to eight weeks of age. In the G-II and G-IV group of birds the IgG level reduced from four to six weeks of age.

4.7 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON PRODUCTION PARAMETERS

4.7.1 Body Weight

The body weight of birds at weekly intervals for the control (provided with a floor space of 696cm^2/bird), stressed control (348cm^2/bird) and overcrowded birds supplemented with feed supplements such as Protexin, virginiamycin and vitamin C from fourth week of age to eighth week of age in summer as well as rainy seasons are given in table 10.

The body weight of four week old birds in the summer (before applying the overcrowding stress) ranged from 825.00± 46.67g in G-V group of birds to 841.67± 46.80g in G-IV group of birds. In the rainy season, the body weight of four week old birds ranged from 827.08 ± 33.35g in birds of G-IV group to 908.33 ± 36.32g in birds of G-I group. The birds in the summer had a significantly ($P \leq 0.05$) lower body weight when compared to those in the rainy season.

The highest body weight (1279.17 ± 47.06g) among the five week old broilers in the summer season was observed in control G-I group of birds and lowest value (1191.67± 53.60g) was noticed in stressed control G-II group of birds. In the rainy season, the body weight was highest (1354.17± 43.72g) in control G-I group of birds and lowest (1220.83± 28.51g) in stressed control G-II group of birds. In the summer, among the five week old birds no significant difference in the body weight was noticed between the different groups of birds, whereas in the rainy season the birds of stressed control G-II group had a body weight value significantly ($P \leq 0.05$) lower than that of unstressed control G-I group. The other groups of broilers (G-III and G-IV) also had the value of body weight significantly ($P \leq 0.05$) lower than the weight of G-I group of birds. Vitamin C supplemented G-V group of birds showed a body weight, which was statistically similar to the body weight of birds in G-I group. On comparing the body weight of five week old birds in summer and rainy seasons, the G-II (stressed control) and G-IV (overcrowded birds supplemented with virginiamycin) group of birds in summer had significantly ($P \leq 0.05$) lower body weight than their counterparts in the rainy season.

Among the six week old broiler chicks in the summer, the body weight varied between 1708.91±45.00 g in control G-I group of birds and 1610.84 ± 41.04g in stressed control G-II group of birds. No significant difference in the body weight was noticed between the different groups of birds. In the rainy season, out of the five groups of broiler chicks, the highest body weight of 1750.00±51.86g was noted in control G-I group of birds and lowest body weight of 1604.17±33.97g was noted in stressed control G-II group of birds. The birds in the G-I (unstressed control) group had a body weight significantly ($P \leq 0.05$) higher than that of G-II (stressed control) group. The body weight of birds in G-III (overcrowded birds supplemented with Protexin) and G-IV (overcrowded birds supplemented with virginiamycin) groups were also significantly ($P \leq 0.05$) lower than that of G-I group. The body weight of birds in G-V (overcrowded birds supplemented with vitamin C) group was between that of G-I and G-II groups of birds. No significant difference in the body weight was noticed between the summer and rainy seasons.

Among the seven week old broiler chicks in the summer, the body weight varied between 2071.41±62.71g in control G-I group of birds and 1940.01±40.36g in stressed control G-II group of birds. The body weight of G-II group of broiler chicks was significantly ($P \leq 0.05$) lower than that of control G-I group and vitamin C supplemented overcrowded chicks of G-V group. The G-III (overcrowded birds supplemented with Protexin) and G-IV (overcrowded birds supplemented with virginiamycin) group of birds had a body weight value between that of control G-I and stressed control G-II group. In the rainy season, among the seven week old broilers, the highest body weight of 2137.50± 50.42g was noticed in control G-I group of birds and lowest body weight of 1987.50± 48.90g was noticed in stressed control G-II group of birds. The birds in the G-I (unstressed control) group had a body weight significantly (P≤0.05) higher than that of G-II (stressed control) group. The Protexin supplemented G-III group of birds had a mean body weight of 1991.67±34.72g which was also significantly (P≤0.05) lower when compared to birds of G-I (unstressed control) group. The G-IV group of birds also had a body weight value (2012.50±54.40g) significantly (P≤0.05) lower when compared to the weight of birds in G-I (unstressed control) group. The overcrowded birds supplemented with vitamin C showed a body weight (2058.33±59.62g) which was neither significantly less than that of G-I group nor significantly higher than the weight in stressed control G-II group.

At eighth week of age the body weight of chicks in summer season ranged from 2219.18±62.60g in G-II group of birds to 2346.41±64.46g in G-I group of birds. The G-I, G-III and G-V groups had the value of body weight significantly (P≤ 0.05) higher than that of G-II group of birds. The overcrowded virginiamycin supplemented G-IV group of chicks was found to have a body weight value (2325.00±41.06g) between that of stressed control G-II group and unstressed control G-I group of birds. In rainy season, the highest body weight (2429.17±47.06g) was seen in control G-I group of birds and lowest body weight (2247.67±56.35g) was seen in stressed control G-II group of birds. The G-I (control) and G-V (overcrowded birds supplemented with vitamin C) group of birds had significantly (P≤0.05) higher body weight when compared to the body weight of G-II (stressed control) group of birds. The birds of G-III and G-IV group had significantly (P≤0.05) lower body weight when compared to birds of G-I and G-V groups.

Table 10 Effect of overcrowding stress and antistress agents on weekly body weight of broiler chicken from fourth to eighth week of age in summer and rainy seasons. Mean ± SE (n=24)

Seasons	Parameter / Weeks / Groups	Body weight (g)/bird/week				
		4	5	6	7	8
S_1	G -I	837.50±39.95^{**a}	1279.17±47.06^{a}	1708.91±45.00^{a}	2071.41±62.71^{a}	2346.41±64.46^{a}
	G -II	833.33±43.66^{**a}	1191.67±53.60^{*a}	1610.84±41.04^{a}	1940.01±40.36^{b}	2219.18±62.60^{b}
	G -III	829.17±35.06^{*a}	1229.17±45.42^{a}	1654.17±48.64^{a}	2025.00±49.43^{ab}	2337.50±50.42^{a}
	G -IV	841.67±46.80^{**a}	1216.67±39.09^{*a}	1639.58±40.76^{a}	2008.33±41.21^{ab}	2325.00±41.06^{ab}
	G -V	825.00±46.67^{**a}	1245.83±48.25^{a}	1671.50±55.43^{a}	2065.28±71.78^{a}	2388.19±68.11^{a}
S_1	G -I	908.33±36.32^{**a}	1354.17±43.72^{a}	1750.00±51.86^{a}	2137.50±50.42^{a}	2429.17±47.06^{a}
	G -II	829.17±29.17^{**a}	1220.83±28.51^{*b}	1604.17±33.97^{b}	1987.50±48.90^{b}	2247.67±56.35^{b}
	G -III	833.33±27.06^{*a}	1237.50±48.51^{b}	1612.50±42.25^{b}	1991.67±34.72^{b}	2291.67±41.67^{b}
	G -IV	827.08±33.35^{**a}	1254.17±53.82^{*b}	1620.83±41.95^{b}	2012.50±54.40^{b}	2291.67±59.93^{b}
	G -V	835.42±24.71^{**a}	1275.00±44.17^{ab}	1679.17±36.65^{ab}	2058.33±59.62^{ab}	2362.50±65.17^{a}

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2),
G -III Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and
G -V Overcrowded and vitamin C supplemented group.
S_1 Summer season. **S_2** Rainy season.
a, b Means within a column with no common superscripts are significantly different at 5% level
* Between seasons significantly different at 5% level
** Between seasons significantly different at 1% level

In summer the overall mean body weight value for four to eight weeks period was also significantly lower in G-II group of birds (1464.17±25.86g) when compared to the overall mean body weight value of G-I (unstressed control) and G-V (overcrowded birds supplemented with vitamin C) group of birds (1575.83±38.42g and 1564.17±40.29g, respectively). The mean body weight value for four to eight weeks period noticed in G-III and G-IV group of birds were not significantly different from that of G-I and G-II birds. The birds of G-III (overcrowded birds supplemented with Protexin) and G-IV (overcrowded birds supplemented with virginiamycin) group had a body weight numerically higher than that of G-II and lower than that of G-I group of chicks. In the rainy season, the overall mean body weight for four to eight weeks period ranged between 1678.33±35.86 g in G-I (unstressed control) group of birds to 1563.33±33.65 g in G-II (stressed control) group of birds. The birds in control G-I group had a body weight value significantly ($P \leq 0.05$) higher than the body weight value of stressed control G-II group of birds. The G-III, G-IV and G-V group of birds had a body weight numerically higher than that of G-II birds and lower than that of G-I birds. The G-II (stressed control) and G-IV (overcrowded birds supplemented with virginiamycin) birds had a body weight significantly lower ($P \leq 0.05$) in summer than those in rainy season.

Among the birds of six, seven and eight weeks of age, even though no significant difference in the body weight was observed between summer and rainy seasons, at all ages the body weight of birds reared in summer was numerically lower than their counterparts in the rainy season.

4.7.2 Body Weight Gain

The weekly body weight gain of the broiler chicks are given in table 11. The results revealed that the body weight gain of broiler chicks from five to eight weeks of age were not affected by the overcrowding in both summer as well as in rainy seasons. However, overcrowding produced a non-significant reduction in the body weight gain.

During summer season, the body weight gain of broiler chicks at fifth week of age was highest (441.67± 43.88 g) in unstressed control birds of G-I group and lowest value of body weight gain (358.33± 40.75g) was noticed in birds of stressed control G-II group. The G-I and G-V groups of birds showed a body weight gain significantly (P≤0.05) higher (441.67±43.88g and 420.83±22.58g, respectively) than the birds of G-II group. In the rainy season, the body weight gain was highest (445.83±17.90g) in G-I group of birds, and lowest (391.67±33.05g) in G-II group of birds. As in the summer, in the rainy season also the body weight gain of birds in G-I and G-V groups were significantly (P≤0.05) higher when compared to the G-II group of birds.

The sixth week body weight gain of birds reared in summer ranged from 419.17 ± 27.84g in birds of stressed control G-II group to 429.74± 27.06g in birds of control G-I group. Among the overcrowded birds supplemented with dietary antistress feed supplements, vitamin C supplemented overcrowded birds of G-V group had the highest body weight gain (425.67±44.52g). However, no significant difference in the body weight gain was noticed between different groups of birds. In the rainy season, the body weight gain of six week old birds was lowest (366.67±34.45g) in virginiamycin supplemented overcrowded birds of the G-IV group. The highest body weight gain (404.17± 24.20g) was seen in the G-V (overcrowded birds supplemented with vitamin C) group of birds. No significant difference in body weight gain was observed between the G-I and G-II groups of birds. Also there was no significant difference in body weight gain between the summer and rainy seasons. However, the birds of G-IV group (overcrowded birds supplemented with virginiamycin) had a body weight gain, which was significantly (P≤0.05) lower than the birds of G-V (overcrowded birds supplemented with vitamin C) group.

Table 11 Effect of overcrowding stress and antistress agents on weekly body weight gain of broiler chicken from fifth to eighth week of age in summer and rainy seasons. Mean ± SE (n=24)

Seasons	Parameter	Body weight gain (g)/bird/week				
	Groups \ Weeks	5	6	7	8	Mean ±SE
S_1	G-I	441.67±43.88 $^{a}_{NS}$	429.74±27.06 $^{a}_{NS}$	362.50±43.57 $^{ab}_{NS}$	275.00±39.65 $^{ab}_{NS}$	377.23±22.69 $^{a}_{NS}$
	G-II	358.33±40.75 $^{b}_{NS}$	419.17±27.84 $^{a}_{NS}$	329.17±29.81 $^{a}_{NS}$	279.17±33.97 $^{b}_{NS}$	346.46±14.47 $^{a}_{NS}$
	G-III	400.00±26.11 $^{ab}_{NS}$	425.00±25.00 $^{a}_{NS}$	370.83±27.15 $^{ab}_{NS}$	312.50±41.80 $^{ac}_{NS}$	377.08±11.60 $^{a}_{NS}$
	G-IV	375.00±25.00 $^{bc}_{NS}$	422.92±36.86 $^{a}_{NS}$	368.75±29.05 $^{ab}_{NS}$	316.67±28.43 $^{ac}_{NS}$	370.84±14.38 $^{a}_{NS}$
	G-V	420.83±22.58 $^{ac}_{NS}$	425.67±44.52 $^{a}_{NS}$	393.75±47.09 $^{b}_{NS}$	322.92±61.20 $^{c}_{NS}$	390.79±18.56 $^{a}_{NS}$
S_2	G-I	445.83±17.90 $^{a}_{NS}$	395.83±28.51 $^{ab}_{NS}$	3 87.50±26.92 $^{a}_{NS}$	291.67±28.76 $^{a}_{NS}$	380.21±16.61 $^{a}_{NS}$
	G-II	391.67±33.05 $^{b}_{NS}$	383.33±44.52 $^{ab}_{NS}$	383.33±29.73 $^{a}_{NS}$	260.17±23.40 $^{b}_{NS}$	354.63±13.69 $^{a}_{NS}$
	G-III	404.17±45.00 $^{bc}_{NS}$	375.00±31.68 $^{ab}_{NS}$	379.17±26.44 $^{a}_{NS}$	300.00±33.14 $^{a}_{NS}$	364.59±11.18 $^{a}_{NS}$
	G-IV	427.08±47.62 $^{ab}_{NS}$	366.67±34.45 $^{a}_{NS}$	391.67±43.01 $^{a}_{NS}$	279.17±23.40 $^{ab}_{NS}$	366.15±14.61 $^{a}_{NS}$
	G-V	439.58±42.36 $^{ac}_{NS}$	404.17±24.20 $^{b}_{NS}$	379.17±40.45 $^{a}_{NS}$	304.17±28.51 $^{a}_{NS}$	381.77±15.81 $^{a}_{NS}$

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2),
G -III Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and
G -V Overcrowded and vitamin C supplemented group.
S_1 Summer season. **S_2** Rainy season.
a,b,c Means within a column with no common superscripts are significantly different at 5% level
NS No significant difference between seasons S_1 and S_2.

The average body weight gain of birds at seventh week of age in summer ranged from 329.17± 29.81g in G-II group of birds to 393.75± 47.09g in G-V group of birds. Even though there was no significant difference in the body weight gain between the G-I and G-II groups of birds, the body weight gain of stressed birds fed with vitamin C had a body weight gain value significantly ($P \leq 0.05$) higher than that of the stressed control G-II group of birds. In the rainy season, the body weight gain of broiler chicks at seventh week of age ranged from 379.17±26.44g in G-III group of birds to 391.67±43.01g in G-IV group of birds. There was no significant difference in the body weight gain observed between the different groups of birds.

At eighth week of age, among different groups of birds in summer the highest (322.92±61.20g) gain in body weight was noted in G-V group and lowest (275.00±39.65g) in G-I group of birds. No significant difference in body weight gain was noticed between the G-I and G-II group of birds. However, the stressed birds fed with the vitamin C had a body weight gain significantly ($P \leq 0.05$) higher than that of unstressed control G-I and stressed control G-II group of birds. In the rainy season, the body weight gain of chicks at eighth week of age varied between 260.17±23.40g in G-II group of birds to 304.17±28.51g in G-V group of birds. The birds of G-I, G-III and G-V groups had a body weight gain significantly ($P \leq 0.05$) higher than that of stressed control G-II group.

The overall mean body weight gain from fifth to eighth week in summer varied from 346.46±14.47 g in G-II group of birds to 390.79±18.56g in G-V group of birds. In the rainy season, the average body weight gain from five to eight weeks varied from 354.63±13.69g in G-II group of birds to 381.77±15.81g in G-V group of birds. In both summer and rainy seasons there was no significant difference in the average body weight gain from five to eight weeks of age.

On comparing the body weight gain of birds in summer with their counter parts in rainy season, no significant difference in body weight gain was noticed between the birds at fifth, sixth, seventh or eighth week indicating that temperature range of 22.83±0.12^{0}C to 32.68±1.17 ^{0}C did not affect the body weight gain in broilers.

4.7.3 Feed Intake

The average weekly feed intake per bird in control and treatment groups along with the overall mean weekly feed intake per bird from five to eight weeks period are displayed in the table 12.

During both the seasons, the feed intake from five to eight weeks period was least in the stressed control G-II group of birds. The highest feed consumption was in unstressed control G-I group of birds. Throughout the period of study, the average weekly feed intake of stressed control G-II group of birds was less than that of unstressed control G-I group of birds.

In summer, among the birds at fifth week of age the feed intake was highest (854.17g) in control G-I group of birds and lowest (741.67g) in stressed control G-II group of birds. The stressed birds supplemented with vitamin C (G-V) had a feed intake value (812.50g) close to that of unstressed control G-I group of birds (854.17g). Eventhough, Protexin and virginiamycin increased the feed consumption of stressed birds the increase was not up to the level of unstressed control G-I group of birds. In the rainy season the feed intake was maximum (875.00g) in G-I group of birds and minimum (791.67g) in G-II group of birds.

At the age of six week, among the birds in the summer season, the feed intake was maximum (958.33g) in G-I group of birds and minimum (836.67g) in G-V group of birds. In the rainy season the highest (1008.33g) feed intake was observed in the birds of G-I group and the lowest (854.17g) in birds of G-III group. In the rainy season also, the antistress supplementations failed to improve the feed intake of six week old stressed birds.

Table 12 Effect of overcrowding stress and antistress agents on weekly feed intake of broiler chicken from fifth to eighth week of age in summer and rainy seasons. Mean ± SE (n=24)

Parameter		Feed intake (g)/bird/week				
Seasons	Groups \ Weeks	5	6	7	8	Mean ±SE
S_1	G -I	854.17	958.33	979.17	945.83	934.38±27.61
	G -II	741.67	908.33	850.00	858.33	839.58±34.44
	G -III	791.70	858.30	904.20	995.80	887.50±42.85
	G -IV	775.00	854.17	900.00	1025.00	888.54±52.30
	G -V	812.50	836.67	929.17	979.17	889.38±39.09
S_2	G -I	875.00	1008.33	1029.17	1029.17	985.42±37.13
	G -II	791.67	916.67	916.67	920.33	886.33±31.57
	G -III	858.33	854.17	900.00	941.67	888.54±20.51
	G -IV	862.50	879.17	925.00	983.33	912.50±27.06
	G -V	866.67	870.83	966.67	983.33	921.88±59.21

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2), **G -III** Overcrowded and Protexin supplemented group, **G -IV** Overcrowded and virginiamycin supplemented group and **G -V** Overcrowded and vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

During summer the feed intake of seven week old birds ranged from 850.00g in G-II group of birds to 979.17g in G-I group of birds. In rainy season the highest feed intake was 1029.17g in G-I group of birds and 900.00g in G-III group of birds. The feed consumption of birds at seventh week was improved by the antistress supplementations. However, in the rainy season the lowest feed intake was observed in G-III group of birds.

At the age of eighth week the feed intake of the birds in summer was maximum (1025g) in G-IV group of birds and minimum (858.33g) in G-II group of birds. In rainy season the feed intake of eight week old birds varied from 920.33g in G-II group of birds to 1029.17g in G-I group of birds. In summer, the overcrowded birds supplemented with the antistress agents had higher feed consumption than the unstressed control G-I group of birds. In rainy season also, the antistress supplementations improved the feed intake of stressed birds.

In both summer and rainy seasons the overall mean feed intake for five to eight weeks period was lowest (839.58g) in stressed control G-II group of birds and highest (934.38g) in unstressed control G-I group of birds. In the rainy season also the highest feed consumption of 985.42g was noticed in G-I group of birds and the lowest value of 886.33g was observed in G-II group of birds.

In all the groups the feed intake was more in the rainy season than in the summer season. Rather than a steady increase in feed intake with age, a fluctuating tendency was noticed in the feed consumption from five week to eight weeks of age.

4.7.4 Cumulative Feed Efficiency (CFE)

The effect of high stocking density (29 birds/m^2) and the effect of antistress feed supplements in the broilers stocked at high density on weekly CFE from five to eight weeks of age in summer and rainy seasons are depicted in table 13.

Table 13 Effect of overcrowding stress and antistress agents on cumulative feed efficiency of broiler chicken from fifth to eighth week of age in summer and rainy seasons. Mean ± SE (n=24)

		Cumulative Feed Efficiency				
Seasons	Weeks / Groups	5	6	7	8	Mean ±SE
S_1	G -I	1.93	2.11	2.29	2.50	2.21±0.12
	G -II	2.07	2.10	2.24	2.42	2.20±0.08
	G -III	1.98	2.00	2.14	2.35	2.12±0.09
	G -IV	2.07	2.04	2.17	2.40	2.17±0.08
	G -V	1.93	1.95	2.08	2.29	2.06±0.08
S_2	G -I	1.96	2.24	2.37	2.59	2.29±0.13
	G -II	2.02	2.20	2.27	2.51	2.25±0.10
	G -III	2.12	2.20	2.26	2.44	2.26±0.07
	G -IV	2.02	2.19	2.25	2.49	2.24±0.10
	G -V	1.97	2.06	2.21	2.41	2.16±0.10

G -I Unstressed control (floor space / bird = 696cm^2), G -II Overcrowded control (floor space / bird = 348cm^2), G -III Overcrowded Protexin supplemented group, G -IV Overcrowded virginiamycin supplemented group and G -V Overcrowded vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

In the summer the stocking density of 29 birds/m^2 reduced the CFE of broilers at fifth week of age when compared to those at 14 birds/m^2. However, the CFE of overcrowded birds (348cm^2/bird) at sixth and eighth weeks of age were superior to the control group (696cm^2/bird).

Result of the study on the effect of antistress dietary supplements on the CFE of overcrowded birds revealed that antistress agents used could improve the CFE of overcrowded birds. Out of the three antistress agents used, vitamin C was found more effective than Protexin and virginiamycin.

In summer, at the age of fifth week the better CFE of 1.93 was noticed in G-I (unstressed control) and G-V (overcrowded birds supplemented with vitamin C) groups of birds and lowest CFE value of 2.07 was observed in stressed control birds of G-II group and virginiamycin supplemented overcrowded birds of G-IV group (2.07). By the age of six weeks, the control G-I group of birds had the lowest CFE (2.11) and vitamin C supplemented G-V group of birds showed the highest CFE (1.95). Among the seven and eight week old broiler chicks also, the better CFE was seen in birds of G-V (overcrowded birds supplemented with vitamin C) group (2.08 and 2.29 respectively) and lowest CFE noticed in birds of G-I (unstressed control) group (2.29 and 2.50 respectively). The overall mean CFE for five to eight weeks period was also better (2.06) in birds of G-V group. The birds of G-II group showed a CFE (mean for 5 to 8 weeks) value of 2.20 as against 2.21 for G-I group of birds

In rainy season, out of the five groups of birds at the fifth week of age, highest value of CFE (1.96) was seen in control G-I group of birds and lowest CFE (2.12) noticed in Protexin supplemented stressed G-III group of birds. Among the six week old birds, the overcrowded birds supplemented with vitamin C (G-V group) exhibited highest CFE (2.06) and the control G-I group of birds had the lowest CFE (2.24). At seventh and eighth week of age also the vitamin C supplemented overcrowded birds of G-V group showed the highest CFE value (2.21and 2.41 respectively) and control G-I group of birds had the lowest CFE (2.37 and 2.59 respectively). As in the summer, in the rainy season also, the overall mean CFE for five to eight weeks period was highest (2.16) in birds of G-V group. The lowest (2.29) CFE was noticed in the unstressed control birds of G-I group.

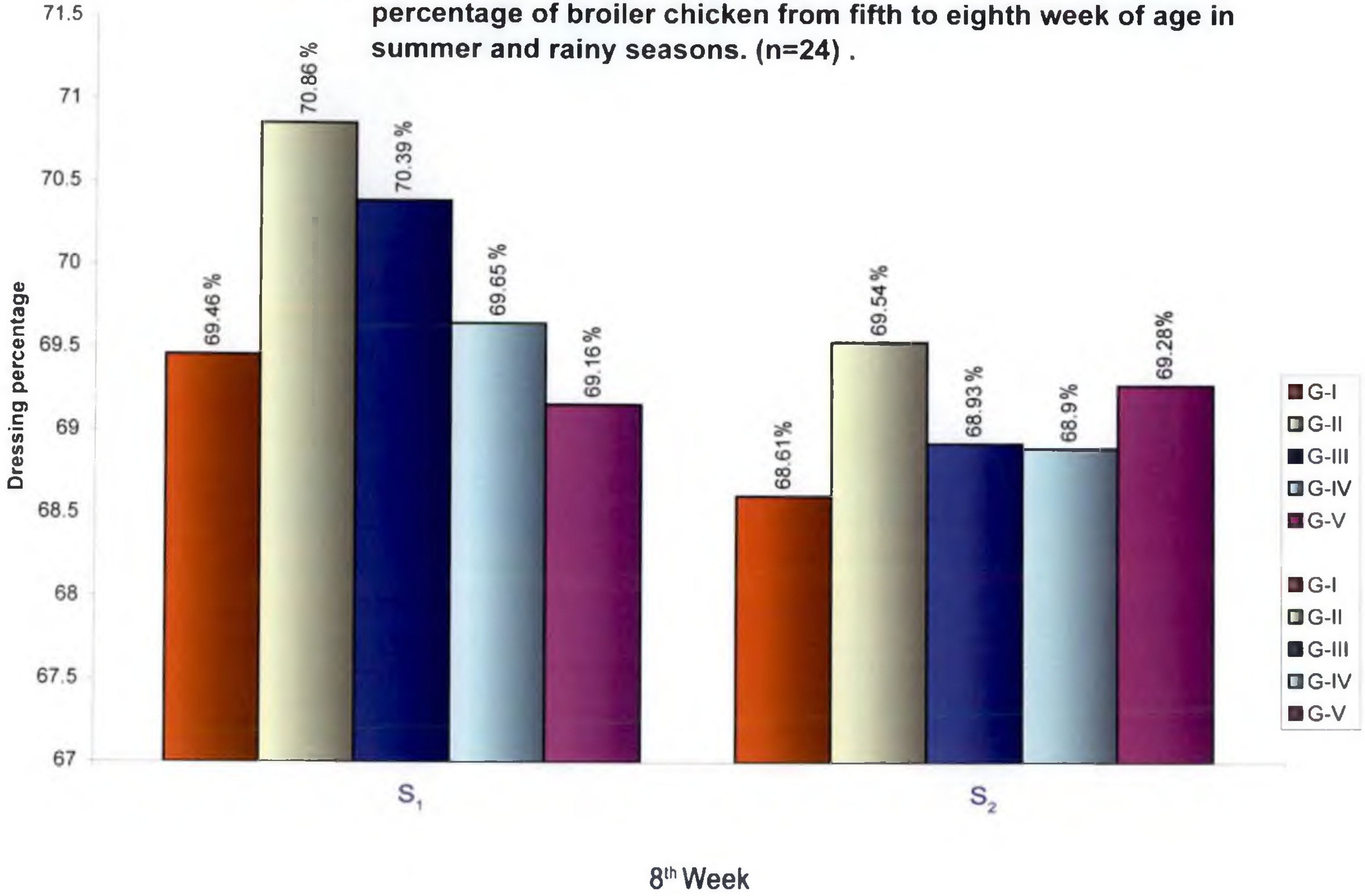
On comparing the CFE value of birds in summer and rainy seasons, the birds in the rainy season had a lower value of CFE than the birds in summer. The CFE of birds in all the groups of both seasons was lowered with age.

4.7.5 Dressing Percentage

The value of dressed weight and dressing percentage in eight week old broiler chicks belonging to control and experimental groups in summer and rainy seasons are shown in figure 11.

Results of the study revealed that floor space reduction had no effect on the dressing percentage of eight week old broilers. The seasons (summer and rainy) also did not produce any significant change in the dressing percentage. The dressed weight of the birds in the summer season ranged from 1572.67±55.68g in birds of G-II group to 1651.67±56.85g in birds of G-V group. In the rainy season the highest (1667.50±22.30g) value of dressed weight was noticed in G-I group of birds and lowest (1563.33±37.86) value was observed in G-II group of birds. The dressing percentage of birds in summer varied from 69.16±0.37 per cent in G-V group of birds to 70.86±0.44 per cent in G-II group of birds. In the rainy season, the dressing percentage varied from 68.61±0.52 per cent in G-I group of birds to 69.54±0.37per cent in G-II group of birds. The stressed control G-II group of birds had the highest dressing percentage in both summer and rainy seasons.

Fig. 11 Effect of overcrowding stress and antistress agents on dressing percentage of broiler chicken from fifth to eighth week of age in summer and rainy seasons. (n=24) .



S_1. Summer Season **S_2. Rainy Season**

4.7.6 Organ Weights

The organ weights of eight week old birds belonging to control and experimental groups of both phases of the study are shown in the table 14.

Overcrowding resulted in no significant difference in weight of any of the organs in the summer and rainy seasons. The fat percentage was also not significantly different between groups or seasons.

In summer the value of heart weight represented as percentage body weight ranged from 0.35±0.02 per cent in G-IV group of birds to 0.37±0.02 per cent in G-II group of birds. In the rainy season the value of heart weight varied from 0.30±0.04 per cent in G-V group of birds to 0.34±0.01 per cent in G-I group of birds. There was no significant difference in the weight of the heart between the different group of birds in both summer and rainy seasons. Similarly, there was no significant difference in weight of the heart between the birds in the summer and those in the rainy season.

The liver weight of birds in summer was between 1.89±0.08 per cent in G-1 group of birds and 1.99±0.11 per cent in G-II group of birds. In rainy season the highest value (1.93±0.08%) of liver weight was in birds of G-I group and lowest (1.63±0.16%) in birds of G-III group. There was no significant difference in weight of liver between different groups of birds during both seasons. The birds in both seasons also had a statistically similar liver weight.

In the summer season the weight of spleen ranged from 0.07±0.01 per cent in G-II group of birds to 0.10±0.01 per cent in G-I group of birds. In the rainy season spleen weight ranged from 0.07±0.01per cent in G-II, G-III and G-IV groups of birds to 0.09±0.01 per cent in G-I group of birds. There was no significant difference in the weight of the spleen between the different groups of birds in both summer and rainy seasons. Similarly, there was no significant difference observed between seasons.

The weight of gizzard in eight week old birds of summer ranged from 1.55±0.06 per cent in birds of G-I group to 1.73±0.05 per cent in birds of G-IV group. In the rainy season, the weight of gizzard ranged from 1.56±0.20 per cent in birds of G-III group to 1.96±0.18 per cent in birds of G-IV group. The gizzard weight was not significantly different between birds of different groups or between birds of two (summer and rainy) seasons.

The fat percentage of birds reared in summer varied from 0.66±0.05 per cent in stressed control G-II group of birds to 0.70±0.05 per cent in birds of G-III group. Even though the fat percentage was not significantly affected by overcrowding the fat percentage in overcrowded birds was numerically less than that of unstressed control birds. However, the Protexin supplemented birds of G-III group had a higher fat content than the unstressed control G-I group of birds.

In the rainy season the fat percentage of birds ranged from 0.70±0.05 per cent in G-I group of birds to 0.78±0.36per cent in G-III group of birds. There was no significant difference in fat percentage observed between the different groups. Unlike the non-significant reduction in fat percentage noticed in overcrowded birds in the summer season, overcrowding did not produce any reduction in fat percentage in the rainy season. The antistress agents did not produce any significant influence on the fat percentage of overcrowded birds in both the summer and rainy seasons.

Table 14 Effect of overcrowding stress and antistress agents on organ weight of broiler chicken at eighth week of age in summer and rainy seasons. Mean ± SE (n=24)

	Groups	Organ weight as percentage body weight (%)				
		Heart	Liver	Spleen	Gizzard	Fat
S_1	G-I	$0.36 \pm 0.02^{a}_{NS}$	$1.89 \pm 0.08^{a}_{NS}$	$0.10 \pm 0.01^{a}_{NS}$	$1.55 \pm 0.06^{a}_{NS}$	$0.69 \pm 0.04^{a}_{NS}$
	G-II	$0.37 \pm 0.02^{a}_{NS}$	$1.99 \pm 0.11^{a}_{NS}$	$0.07 \pm 0.01^{a}_{NS}$	$1.72 \pm 0.09^{a}_{NS}$	$0.66 \pm 0.05^{a}_{NS}$
	G-III	$0.36 \pm 0.02^{a}_{NS}$	$1.94 \pm 0.07^{a}_{NS}$	$0.08 \pm 0.01^{a}_{NS}$	$1.67 \pm 0.08^{a}_{NS}$	$0.70 \pm 0.05^{a}_{NS}$
	G-IV	$0.35 \pm 0.02^{a}_{NS}$	$1.95 \pm 0.07^{a}_{NS}$	$0.08 \pm 0.01^{a}_{NS}$	$1.73 \pm 0.05^{a}_{NS}$	$0.66 \pm 0.07^{a}_{NS}$
	G-V	$0.36 \pm 0.01^{a}_{NS}$	$1.92 \pm 0.06^{a}_{NS}$	$0.08 \pm 0.01^{a}_{NS}$	$1.70 \pm 0.08^{a}_{NS}$	$0.67 \pm 0.04^{a}_{NS}$
S_2	G-I	$0.34 \pm 0.01^{a}_{NS}$	$1.93 \pm 0.08^{a}_{NS}$	$0.09 \pm 0.01^{a}_{NS}$	$1.76 \pm 0.10^{a}_{NS}$	$0.70 \pm 0.05^{a}_{NS}$
	G-II	$0.31 \pm 0.02^{a}_{NS}$	$1.83 \pm 0.07^{a}_{NS}$	$0.07 \pm 0.01^{a}_{NS}$	$1.71 \pm 0.08^{a}_{NS}$	$0.73 \pm 0.06^{a}_{NS}$
	G-III	$0.32 \pm 0.03^{a}_{NS}$	$1.63 \pm 0.16^{a}_{NS}$	$0.07 \pm 0.01^{a}_{NS}$	$1.56 \pm 0.20^{a}_{NS}$	$0.78 \pm 0.36^{a}_{NS}$
	G-IV	$0.32 \pm 0.03^{a}_{NS}$	$1.78 \pm 0.18^{a}_{NS}$	$0.07 \pm 0.01^{a}_{NS}$	$1.96 \pm 0.18^{a}_{NS}$	$0.72 \pm 0.07^{a}_{NS}$
	G-V	$0.30 \pm 0.04^{a}_{NS}$	$1.68 \pm 0.15^{a}_{NS}$	$0.08 \pm 0.01^{a}_{NS}$	$1.82 \pm 0.20^{a}_{NS}$	$0.77 \pm 0.08^{a}_{NS}$

G -I Unstressed control (floor space / bird = 696cm^2), **G -II** Overcrowded control (floor space / bird = 348cm^2), **G -III** overcrowded Protexin supplemented group, **G -IV** Overcrowded virginiamycin supplemented group and **G -V** Overcrowded vitamin C supplemented group

S_1 Summer season. **S_2** Rainy season.

Means within a column with common superscripts did not significantly differ at 5% level

NS Non-significant

4.7.7 Economics of Production

The economics of rearing of broiler chicken with and without applied floor space reduction stress as well as with addition of antistress feed supplements such as Protexin, virginiamycin and vitamin C in stressed birds are given in table 15.

In summer the cost of finisher ration was rupees 37.38; 33.58; 35.50; 35.54 and 35.58 for G-I, G-II, G-III, G-IV and G-V group of birds respectively. In the rainy season the values were 39.42; 35.45; 35.54; 36.50 and 36.88 respectively. The total cost of rearing in summer varied from rupees 71.68 in G-II group of birds to rupees 75.84 in G-III group of birds. In the rainy season, the cost of production ranged from rupees 75.55 in birds of G-II group to 79.52 in birds of G-I group.

During the summer season, the net profit per kg of live weight was maximum (Rs.3.26) in G-V group and minimum (Rs.1.80) in G-II group. In the rainy season the maximum (Rs.2.37) profit was in birds of G-V group and minimum in (Rs.1.38) in G-II group of birds.

Table 15 Cost benefit analysis as influenced by overcrowding stress and antistress agents in eight week old broilers in summer and rainy seasons

SEASONS	S_1					S_2				
GROUPS	G-I	G-II	G-III	G-IV	G-V	G-I	G-II	G-III	G-IV	G-V
Live body weight (g)	2346.41	2219.18	2337.50	2325.00	2388.19	2429.17	2247.67	2291.67	2291.67	2362.50
starter feed intake/bird (g)	1800	1800	1800	1800	1800	1800	1800	1800	1800	1800
Finisher feed intake/bird(g)	3737.5	3358.3	3550	3554.2	3557.5	3941.7	3545.3	3554.2	3650	3687.5
Starter feed cost/kg feed (Rs)	12	12	12	12	12	12	12	12	12	12
Finisher feed cost/kg feed (Rs)	10	10	10	10	10	10	10	10	10	10
Total starter feed cost (Rs)	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6
Total finisher feed cost (Rs)	37.38	33.58	35.50	35.54	35.58	39.42	35.45	35.54	36.50	36.88
Cost of feed additives incorporated (Rs)	0	0	0.24	.0075	0.12	0	0	0.25	0.008	0.12
Total finisher feed cost (Rs)	37.38	33.58	35.74	35.55	35.70	39.42	35.45	35.79	36.51	37.00
Total feed cost (Rs)	58.98	55.18	57.34	57.15	57.30	61.02	57.05	57.39	58.10	58.60
Chick cost (Rs)	14.50	14.50	14.50	14.50	14.50	14.50	14.50	14.50	14.50	14.50
Miscellaneous cost /bird. (Rs)	4	4	4	4	4	4	4	4	4	4
Total cost /bird. Rs.	75.48	71.68	75.84	75.65	75.8	79.52	75.55	75.89	76.6	77.1
Cost of 1 kg meat	38	38	38	38	38	38	38	38	38	38
Return from one bird	89.16	84.33	88.83	88.35	90.75	92.31	85.41	87.08	87.08	89775
Net profit / bird	6.64	5.99	7.97	7.7	9.79	7.50	5.12	6.32	5.61	7.59
Net profit / kg live weight	2.83	1.80	2.56	2.46	3.26	2.26	1.38	1.88	1.57	2.37

G -I Unstressed control (floor space / bird = 696cm^2), G -II Overcrowded control (floor space / bird = 348cm^2), G -III Overcrowded and Protexin supplemented group, G -IV Overcrowded and virginiamycin supplemented group and G -V Overcrowded and vitamin C supplemented group.

S_1 Summer season. S_2 Rainy season.

Discussion

5. DISCUSSION

5.1 CLIMATIC DATA

The data regarding the microclimate inside the experimental cages (Table 4.1 and 4.2) showed that, in summer the mean maximum temperature was lowest (29.30±0.90^{0}C) during the seventh week of the trial and highest (35.10±0.30°C) during second and fourth week. The mean maximum temperature showed a gradual increase from first to second week, then declined by a value of 1.3^{0}C at third week, then increased again at fourth week to reach a maximum temperature at fourth week. Thereafter the temperature gradually decreased. Unlike the mean maximum temperature, which showed a gradual increase from first to third week, the mean minimum temperature increased from second to fourth week to have a maximum value of 26.10±0.30°C at fourth week and then declined from fifth to seventh week. At eighth week the mean maximum and minimum temperature increased by a factor of 2.3°C and 0.5°C respectively. The mean maximum relative humidity (RH) in the forenoon and afternoon were 91.60±1.70 per cent and 81.90±3.90 per cent respectively observed at seventh week of the experiment. The mean rainfall recorded during the summer was 13.50±5.19mm (summer rain). The maximum rainfall of 30.00±10.70mm was noticed at seventh week of the trial.

In the rainy season, the lowest 28.60±0.60°C value of mean maximum temperature was recorded during the fifth week of the trial and the highest (30.80^{0}C±0.20) during the second week. The mean maximum temperature showed a gradual increase from first to second week to have a maximum value at second week, then declined, and increased again at fourth week. Thereafter the temperature reduced by 1.30°C at fifth week, which was the lowest value of recorded mean maximum temperature during the trial period. The mean minimum temperature fluctuated between 22.70±0.34°C and 23.40±0.20°C. The mean maximum RH in the forenoon and afternoon were 94.40±0.40 per cent (at sixth week) and 82.30±0.34 per cent (at eighth week). The mean rainfall obtained in the experimental period of five to eight weeks was 19.03±5.21mm and the maximum recorded rainfall was 34.60±17.35mm when the birds were at eighth week of age. The meteorological data revealed that climatogram of the locality fell within the hot humid climate.

5.2 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON HAEMATOLOGICAL PARAMETERS

5.2.1 Total Erythrocyte Count (TEC)

The study on the effect of overcrowding (348cm^2/bird) stress and antistress agents (Protexin, virginiamycin and vitamin C) on total erythrocyte count of broiler chicks at fourth, sixth and eighth week of age in summer and rainy seasons indicated that high stocking density of 29 birds/m^2 did not produce any significant difference in TEC at sixth and eighth week of age in both summer and rainy seasons.

Reddy (2003) could not observe any significant change in the value of TEC in six and eight weeks old broilers under a stocking density of 22 birds/m^2. The result of the present study and that of earlier reports of Reddy (2003) showed that stocking density had no influence in the TEC value of broiler chicken both at sixth and eighth week of age. According to Zulkifli and Siegel (1995) and Reddy (2003), the floor space reduction being a chronic stress did not produce any discernible effect on TEC. The results of the study and that of previous reports revealed that TEC could not be taken as an indicator of stress, especially in chronic stress like high stocking density.

There was a significant ($P \leq 0.05$) decrease in TEC in eight week old stressed birds supplemented with virginiamycin when compared to overcrowded birds supplemented with vitamin C. However, in both groups the mean TEC value was analogous with the normal range of TEC value for chicken as reported by Sturkie (1965). Odunsi and Onifade (1998) and Odunsi *et al.* (1999) reported that in broilers supplemented with virginiamycin (10mg/100kg feed), the haematological values varied within the normal range. Ali *et al.* (2005) also observed a reduced value of TEC in baby chicks (1-5 days) treated with antibiotics, but in older birds (22-27 days) the values observed were within the normal range. The results of this study are in agreement with the previous reports.

At eighth week of age, the value of TEC in stressed birds supplemented with vitamin C (G-V) in both summer and rainy seasons were more than the TEC value observed in unstressed controls (G-I). So it could be inferred that vitamin C supplementation at the rate of 300mg/100kg feed could promote erythropoiesis and the TEC in eight week old broiler chicks even under the high stocking density (29 birds/m^2) in both summer and rainy seasons. Sahota *et al.* (1994) reported that ascorbic acid supplementation at the rate of 50 to 100mg/kg feed significantly improved the erythrocyte count in chicken exposed to heat stress. In this study, even though the RBC count was highest in overcrowded birds supplemented with vitamin C (G-V), the increase was not significant. This shows that even though vitamin C produced a significant increase in TEC in heat stressed broilers it did not have a significant effect on TEC in overcrowded birds. However, in the present study the vitamin C proved as an antistress agent in stressed (overcrowded) birds.

There was no significant difference in the TEC value between the birds reared in summer and rainy seasons. However, the study indicated that in all the groups the TEC value was higher in the rainy season. This may be attributed to the reduced erythropoiesis in heat stress or may be due to haemodilution, which is an adaptive response to heat stress (Borges *et al.*, 2004).

The present study also suggested that the stocking density of 29 birds/m^2 had no adverse effect on TEC in six and eight weeks old broilers in both summer and rainy seasons. Also, the stress of summer due to heat and high RH did not produce any significant reduction in the TEC value of four, six and eight weeks old broilers. As per the results of the study, vitamin C supplementation at the rate of 300mg per cent level in the diet of overcrowded birds are beneficial whereas, antibiotic (virginiamycin) supplementation had an adverse effect on the erythrocyte count of overcrowded birds.

5 2.2 Haemoglobin (Hb) Concentration

The results of the study on the effect of overcrowding (348cm^2/bird) stress and antistress agents (Protexin, virginiamycin and vitamin C) in overcrowded birds on Hb concentration of broiler chicks at fourth, sixth and eighth week of age in summer and rainy seasons indicated that either in summer or in rainy season overcrowding (348cm^2/bird) did not produce any significant change in the Hb level in six and eight weeks old broilers. The antistress agents (probiotic, virginiamycin and vitamin C) produced no significant impact on Hb concentration of stressed birds.

Emre *et al.* (1991) could not observe any significant change in the Hb level in 40 and 49 days old broilers at stocking densities of 10, 14, 18 and 20 chicks/m^2. Reddy (2003) also did not observe any significant change in Hb concentration in 42 and 56 days old broilers under a stocking density of 22 birds/m^2. Results of the present study reinforce the earlier observations. In this study, a stocking density of 29 birds/m^2 produced no significant change in the Hb concentration.

In rainy season the Hb concentration of eight week old broilers in G-IV group were significantly low when compared to control birds (G-I) and overcrowded birds supplemented with vitamin C (G-V). From this result it was evident that use of virginiamycin as an antisress agent was harmful to the broilers with respect to Hb concentration. On the other hand vitamin C can be very effectively used to improve the haemoglobin concentration in overcrowded broilers. The higher Hb concentration noticed in G-V group of birds were in accordance with the higher TEC value observed in this group of birds.

The results of the experiment showed that, the virginiamycin supplementation reduced the Hb concentration in broilers under overcrowding stress. That is, virginiamycin has an adverse effect on Hb concentration in overcrowded broilers at six and eight weeks of age. The virginiamycin fed stressed birds had the lowest VPRC and TEC. The lowest Hb concentration observed in the birds of stressed group supplemented with virginiamycin (G-IV) can be due to the reduced erythropoiesis. The lowest value of Hb observed in G-IV group was in agreement with the results of Burke and Culha (2003), who recorded a lower Hb level in antibiotic supplemented birds. Ali *et al.* (2005) also noticed a lower Hb level of 6.2g/dl in birds supplemented with antibiotics as against 8.9g/dl in control group.

Seasonal comparison of Hb level revealed that in summer a significant reduction in the Hb concentration resulted in birds of all the groups studied (G-I, G-II, G-III, G-IV and G-V). These results are in support of earlier reports that heat stress reduced the Hb levels in birds (Donkoh, 1989; Furlan *et al.*, 1999; Borges *et al.*, 2004). However, Borges *et al.* (1999) was of the opinion that heat stress increased the Hb concentration in birds, which is contrary to the present observations. According to Luger *et al.* (2003), the development of mature peripheral red blood cells from pluripotent stem cells in the bone marrow is a complex process regulated by erythropoietin, corticosterone, triiodothyronine (T_3) and growth factors. Wessely *et al.* (1997) reported that the glucocorticoid hormone is a key regulator for self-renewal of erythroid progenitors. The glucocorticoids released as a result of the stress might have reduced the eythropoiesis. Mejo (2006) observed a significant increase in Hb content in untreated five days heat stressed cockerels which later decreased to reach the basal line in 10 days heat stressed cockerels. The reduced Hb noticed in summer might be the result of long term exposure of birds to heat stress. Antistress agents used in the study failed to improve the Hb level in heat stressed birds.

No previous reports on the combined effect of heat stress, overcrowding stress and antibiotics are available to compare the low values of Hb noticed in overcrowded virginiamycin supplemented group of birds in summer. Even then it may be concluded that the lowest Hb value observed in antibiotic supplemented overcrowded birds in G-IV group in summer may be due to the combined effect of heat, overcrowding and antibiotic.

5.2.3 Volume of Packed Red Blood Cells (VPRC)

Analysis of the results on the effect of overcrowding stress and antistress agents on the VPRC value of six and eight weeks old broilers in summer and rainy seasons revealed that the VPRC value of four week old birds in different groups in either seasons did not differ significantly.

The overcrowding stress did not produce any significant effect on the value of haematocrit in both six and eight weeks old broiler chicks. The observations of the study was in agreement with Emre *et al.* (1991) who reported that no significant change occur in the haematocrit value of 40 and 49 days old broiler chicks stocked at densities of 10, 14, 18 and 20 chicks/m^2. Reddy (2003) also could not observe any significant change in the value of VPRC in broiler chicken at a stocking density of 22 chicks/m^2. The results of the present experiment with a stocking density of 29 birds/m^2 did not produce any significant change in VPRC value of broilers at four and six weeks of age.

A significantly ($P \leq 0.05$) reduced VPRC value was noticed in six and eight week old birds of G-IV group (overcrowded birds supplemented with virginiamycin) in summer when compared to the control birds. However, the significant reduction was observed only at sixth week during the rainy season. It is apparent from the present study that antibiotics adversely affected VPRC level in overcrowded (29 birds/m^2) birds in summer as well as in rainy seasons. Dash *et al.* (1992) also observed a non-significant reduction in VPRC value of broilers fed with virginiamycin at the rate of 10mg/100 kg feed. According to Ali *et al.* (2005), the erythrocytes were microcytic in birds treated with antibiotics.

Yahav *et al.* (1997) reported a significant reduction in PCV value of broilers on chronic exposure to high temperature. The prolonged and significantly ($P \leq 0.05$) reduced VPRC value noticed in G-IV group of birds in summer might be due to the combined effect of heat, overcrowding and antibiotic rather than due to the overcrowding stress alone. The reduced VPRC noted in birds of G-IV group were in accordance with the reduced erythrocyte count observed in the group. In summer, the VPRC value in probiotic and vitamin C supplemented overcrowded birds were very close to the VPRC level of unstressed control (G-I) group whereas in stressed control birds of G-II group the value was less (nonsignificant) than that of G III (overcrowded birds supplemented with Protexin) and G-V (overcrowded birds supplemented with vitamin C) groups. This indicated that, probiotic and vitamin C are equally effective in improving the VPRC level of heat stressed overcrowded birds.

Mohan *et al.* (1996) did not find any significant ($P \leq 0.05$) change in the VPRC value on dietary supplementation of probiotic. Even though the results of the present study support the earlier findings, it is clear from the results of the study that the probiotics and vitamin C produced beneficial effect in improving the VPRC value in six and eight week old broilers reared under reduced floor space during the summer season.

Results clearly indicated that overcrowding (29 birds/m^2) could produce a numerical reduction in VPRC value in six and eight weeks old broilers in both summer and rainy seasons. Even though the Protexin and vitamin C could effectively improve the VPRC value in overcrowded birds, virginiamycin was harmful to the stressed birds as far as VPRC value is concerned. Reports for comparing the effect of supplementation of vitamin C and Protexin on birds subjected to different seasons and floor space reduction are meagre.

Earlier reports regarding the haematocrit values in broilers under heat stress are inconsistent. Yahav *et al.* (1997) observed a significant ($P \leq 0.05$) reduction in the value of VPRC in birds reared at higher temperature (30 and

35^0C) while acute exposure of birds to high temperature did not have any significant effect on PCV. Zhou *et al.* (1998a) observed a significant reduction in VPRC value between a temperature of 20 and 30°C and no significant change in VPRC between 30 and 35°C. Furlan *et al.* (1999) reported that acute heat stress was associated with a reduction in VPRC value. On the other hand Altan *et al.* (2000) found that haematocrit values were not affected by heat stress (38±1°C) in 35 days old broilers.

Results of the present study paralleled earlier findings of Yahav *et al.* (1997), Zhou *et al.* (1998a) and Furlan *et al.* (1999) and the reduction in VPRC may be due to the reduced erythropoiesis in summer or due to the haemodilution, an adaptive response to heat stress as reported by Darre and Harrison (1987) and Borges (2004) or a combined effect of both.

The antistress agents (probiotic, virginiamycin and vitamin C) supplemented could not remove the harmful effects of heat stress indicated by the low VPRC levels noticed in all groups of birds during the summer season.

5.2.4 Erythrocyte Indices

5.2.4.1 Mean Corpuscular Volume (MCV)

The results of the investigation vide table 5.2 clearly indicated that the stress due to floor space reduction did not produce any significant change in the MCV value of six and eight weeks old birds. However, there was a nonsignificant reduction in the size of the erythrocytes indicated by low MCV value. The antistress agents like probiotic and vitamin C were found to be useful in improving the MCV value of overcrowded birds in the summer. On the other hand, virginiamycin produced an adverse effect on MCV value in overcrowded heat stressed birds (G- IV) at six and eight weeks of age. . In the rainy season, virginiamycin did not produce any adverse effect on the MCV value in overcrowded birds at both six and eight weeks of age (Table 5.2). In the rainy season all the three feed supplements were equally effective in improving the value of MCV. The reduction in the MCV value was the effect of overcrowding which was higher in the summer season than in the rainy season.

Significantly ($P \leq 0.05$) low MCV value noticed in G-IV (overcrowded birds supplemented with virginiamycin) group of birds in summer may be due to the combined effect of heat stress, overcrowding stress and antibiotic rather than the impact of one alone. Hill (1983) opined that all kinds of stressors were believed to produce same physiological response in animals as well as in birds. Burke and Culha (2003) and Ali *et al.* (2005) reported that microcytosis occurred in antibiotic treated broilers, which supported the results of the current study.

Results of the present study suggested that overcrowding ($348 cm^2$/bird) would not produce any significant effect on the MCV value of six and eight weeks old broiler chicks in summer and rainy seasons. Paralleled with the observations of the present study, Reddy (2003) did not observe any significant ($P > 0.05$) change in MCV value in six and eight weeks old broiler chicks under overcrowding stress ($492 cm^2$/bird). From the results of the present study and previous studies it may be concluded that stress had no significant impact on the MCV value of birds. However, it could produce a mild microcytosis, which was nonsignificant. Antistress supplements did not produce any significant effect on value of MCV in overcrowded birds.

5.2.4.2 Mean Corpuscular Haemoglobin (MCH)

The analysis of the results obtained in the study on the impact of overcrowding stress and the effect of dietary supplements Protexin, virginiamycin and vitamin C on MCH value of six and eight weeks old broiler chicks in summer and rainy seasons showed that the overcrowding stress did not have any significant ($P > 0.05$) effect on the MCH value of six and eight weeks old broiler chicks in both summer and rainy seasons. As per the results of the experiment, the age (4^{th}, 6^{th} and 8^{th} week) as well as season (summer and rainy season) produced no significant (P>0.05) impact on the MCH value of the birds. The dietary supplements viz., Protexin, virginiamycin and vitamin C proved to have no significant (P>0.05) effect on the MCH value of overcrowded birds at six and eight weeks of age in both seasons.

Even though no significant (P>0.05) difference was noticed between the groups, the virginiamycin supplemented birds of G-IV group showed a numerically low MCH value at six and eight weeks of age in summer and at eighth week of age in rainy season. This indicated that virginiamycin produced an adverse effect on the MCH value in overcrowded birds. This adverse effect was more evident in summer than in rainy season (Table 5.2).

Analysis of the effects of virginiamycin on the values of Hb, MCV and MCH (Table 5.1 and 5.2) showed that virginiamycin had an adverse effect on Hb, MCV as well as MCH value in overcrowded birds. However, the value obtained in the virginiamycin fed overcrowded birds compared favorably well with normal values as reported by Swenson and Reece (1996).

The MCH value in different groups did not vary between the two seasons. However, a low MCH value was observed in all groups in summer when compared to that in rainy season except in stressed control G-II group of birds at sixth week of age.

From the results of the study, it may be concluded that stocking density of 29 birds/m^2 did not produce any significant (P>0.05) change in the MCH value of six and eight weeks old broiler chicks in summer and rainy seasons. Similar to the observations in this study, Reddy (2003) also did not observe any significant impact for the overcrowding stress (22 birds/m^2) on MCH value in six and eight weeks old broilers. The lack of significant change suggested that MCH value is not an indicator of stress response in birds.

5.2.4.3 Mean Corpuscular Haemoglobin Concentration (MCHC)

The results of the study on the effect of overcrowding stress and antistress dietary supplements on the MCHC value of six and eight weeks old broiler chicks in summer and rainy seasons revealed that overcrowding stress (348cm^2/bird) did not produce any significant (P>0.05) effect on the value of MCHC in six and eight weeks old broiler chicks in summer as well as rainy seasons. The dietary supplementations (Protexin, virginiamycin and vitamin C) used were found to have no significant (P>0.05) influence on the value of MCHC in six and eight weeks old broiler birds under trial. However, the use of probiotic Protexin during summer and antibiotic virginiamycin during rainy season resulted in slight reduction in the MCHC value. From the study, it was seen that age of birds (between 4 to 8 weeks) had no significant (P>0.05) impact on the MCHC value of broilers.

According to the reports of Reddy (2003), overcrowding stress (33% reduction in floor space) had no significant (P>0.05) effect on the MCHC value of six and eight week old broiler chicks. Also vitamin C supplementation produced no significant (P>0.05) change in the MCHC value of six and eight weeks old birds under overcrowding stress. As per the results of the present study 50 per cent reduction in floor space so as to have a space allowance of 348cm^2/bird also resulted in no significant (P>0.05) variation in the MCHC value in six and eight weeks old birds either in summer or in rainy season. Results of the present study and that of Reddy (2003) showed that MCHC is not an indicator of stress in broilers.

5.2.5 Total Leukocyte Count (TLC)

The results of the present experiment on the effect of stress due to floor space reduction (50% reduction in the floor space) and effect of dietary supplements such as virginiamycin, Protexin and vitamin C on the TLC value of six and eight weeks old broilers in summer and rainy seasons revealed that, leukocyte count value was not significantly (P>0.05) affected by the applied floor space reduction. The antistress feed supplements did not produce any significant (P>0.05) effect on the TLC value of six and eight weeks old broiler chicks in both summer and rainy seasons.

The results of the study indicated that, either the age or the season did not have any significant (P>0.05) influence on the TLC count. However, a numerically low value of TLC was noticed in the stressed control birds of G-II group in both summer and rainy seasons. Among the treatment groups of birds (G-III, G-IV and G-V) vitamin C supplementation produced a positive response in the TLC value in overcrowded birds on both seasons.

Emre *et al.* (1991) reported a high leukocyte count in six week old broiler chicks kept at a stocking density of 18 or 22 birds/m^2 when compared to those kept at 10 or 14 birds/m^2. On the other hand, Reddy (2003) did not observe any significant increase in leukocyte count of six and eight weeks old broiler chicks at a stocking density of either 14 birds/m^2 or 22 birds/m^2. In the present experiment also, a stocking density of 29 birds/m^2 did not produce any significant change in the TLC value of six and eight weeks old broilers in both summer and rainy seasons when compared to those kept at a stocking density of 14 birds/m^2. The results of the present study are in consonance with the results of Reddy (2003). The diversity between the results of the present study and that of Emre *et al.* (1991) may be due to the difference in breed of the bird and the variations in the other environmental stressors, which influence the TLC value.

The study revealed that the TLC value of birds in summer was numerically less than their counter parts in rainy season. Thaxton *et al.* (1968) and Nathen *et al.* (1976) reported reduced leukocyte count in heat stressed chicken. Mashaly (2004) also observed lower leukocyte count in birds exposed to a temperature of 35^0C when compared to those at 23.9^0C. The lower TLC values observed in the experiment were in support of earlier findings. The prolonged activation of hypothalamo-hypophyseal–adrenal (HPA) axis during heat stress would have resulted in increased corticosteroid release, leading to immunosuppression and related leucocytopenia as reported by Graczyk *et al.* (2003b). The higher temperature to which birds were exposed before the experimental period might have resulted in an adaptive response resulting in a numerical rather than significant decrease in the TLC value noticed in the trial. The data obtained in the present study reinforced the opinion of Grey *et al.* (1989) that leukocyte count was an inconsistent and unreliable indicator of stress in chicken.

5.2.6 Differential Leukocyte Count (DLC)

5.2.6.1 Monocyte Count

As per the results of the present study the stress due to overcrowding did not produce any significant effect on the monocyte count of six and eight weeks old birds in summer and rainy seasons. The age or season did not produce any significant change in the percentage of monocyte in control or treatment group of birds.

Maxwell *et al.* (1990a), Maxwell (1993) and Reddy (2003) also reported that there was no significant change in the monocyte count in stressed birds as compared with unstressed birds. However, Mc Farlane *et al.* (1989) reported high monocyte count in birds subjected to continuous sound stress (80-95db). The observations of the study agreed with the reports of Maxwell *et al.* (1990a), Maxwell (1993) and Reddy (2003).

The lack of significant change in the percentage of monocyte between summer and rainy seasons suggested that temperature range of 22.83^{0}C to 32.68^{0}C did not have any significant impact on the monocyte count.

Results of the study suggested that monocyte count is an unreliable indicator of stress especially in chronic stress like overcrowding in birds.

5.2.6.2 Basophil Count

Results of the present study showed that overcrowding produced a significantly ($P \leq 0.05$) high basophil count in six and eight weeks old broilers in summer and rainy seasons.

The dietary antistress agents used were not effective in bringing down the basophil count in overcrowded birds. All the dietary antistress agents reduced the basophilia in overcrowded birds. However, the reduction was not significant ($P>0.05$). Protexin and virginiamycin were found better than vitamin C in reducing the basophilia.

Except a significant ($P \leq 0.05$) increase in the basophil count noticed between four and six weeks old birds in summer no significant ($P>0.05$) change in the basophil count was observed between the birds under different age groups. Seasons (summer and rainy) also did not produce any significant ($P>0.05$) effect on the basophil count of control or treatment groups of birds. Observations in the present study agreed with the findings of Maxwell (1993), Maxwell and Robertson (1995) and Reddy (2003) where basophilia was observed in stressed birds, which was attributed as a response to severe stress. According to Maxwell and Robertson (1995), in extreme stress conditions both heterophilia and basophilia were common. Along with the previous reports the results of the present study (heterophilia and basophilia) suggested that overcrowding (29 birds/m^2) produced severe stress in caged birds at sixth and eighth week of age.

5.2.6.3 Eosinophil Count

The results of the study indicated that overcrowding produced a significant ($P \leq 0.05$) increase in the eosinophil count in six week old birds in the summer. However, the stress due to reduced floor space (50% reduction in floor space) did not have any significant ($P > 0.05$) effect on eosinophil count of eight week old birds in the summer. In the rainy season, overcrowding did not produce any significant change in eosinophil count of six or eight week old birds. There was no change in the eosinophil count of birds at different age groups (from 4 to 8 weeks period), which proved that age did not influence the eosinophil count in birds.

Grey *et al.* (1989) suggested that eosinophil counts were inconsistent and unreliable indicators of stress response in birds. Reddy (2003) could not observe any significant change in eosinophil count in six and eight weeks old birds at a floor space of $734 cm^2$/bird and $492 cm^2$/bird.

Results of the study are well in agreement with the earlier reports. From this study it is also clear that temperature range of 22.83 ± 0.12^0C to 32.68 ± 1.17^0C had no significant effect on the eosinophil count of six and eight weeks old caged broilers.

Observations of the study reinforce the statement of Grey *et al.* (1989) that eosinophil count is an inconsistent and unreliable indicator of stress response in birds.

5.2.6.4 Heterophil Count

The results of the study indicated that overcrowding produced heterophilia in both six and eight weeks old broiler chicks in summer and rainy seasons. Grey *et al.* (1989) reported that absolute heterophil count was one of the most sensitive haematopoietic indicators of chronic stress. Kuan *et al.* (1990), Prabhakaran *et al.* (1997) and Puvadolpirod and Thaxton (2000a) reported heterophilia in birds under various stress conditions. Reddy (2003) noticed heterophilia in six and eight weeks old broiler chicks under overcrowding stress (22 birds/m^2).

The observations in the present study were in consonance with the earlier reports. However, it disagreed with the report of Patterson and Siegel (1998) where increasing the stocking density did not affect the heterophil count in birds. Along with the previous reports, the observations in the present study indicated that housing density of 29 birds/m^2 is stressful to the birds and overcrowding for two weeks can be considered as a chronic stress.

From the highly significant ($P \leq 0.01$) value of heterophil count observed in unstressed control G-I group of birds in summer at sixth and eighth week of age, it could be suggested that stress due to the heat along with the high humidity in summer months is highly stressful to the birds.

The antistress feed supplements used in the study were all effective in reducing the heterophilia in the overcrowded birds in summer than in rainy season. From this it could be assumed that the feed supplements (Protexin, virginiamycin and vitamin C) were more efficient in reducing the heterophilia due to the combined effect of overcrowding and heat rather than the overcrowding stress alone. Out of the three antistress agents vitamin C was found to be better (as the vitamin C supplemented stressed birds showed a heterophil count near to that of unstressed controls) than the other two feed supplements.

Pardue and Thaxton (1986) reported that ascorbic acid could reduce the synthesis of glucocorticoids in birds under stress. The reduced glucocorticoids in ascorbic acid supplemented birds might be the cause of reduced heterophil count noticed in the ascorbic acid supplemented G-V group of birds.

The analysis of the result showed that a positive correlation existed with the age and heterophil count of birds within a group from four to eight weeks of age since the values of all groups increased with the age.

5.2.6.5 Lymphocyte Count

The results of the study on the effect of floor space reduction stress (50% reduction in floor space) and the effect of feed supplements on six and eight weeks old overcrowded broiler chicken in summer and rainy seasons showed that floor space reduction resulted in a significantly ($P \leq 0.05$) reduced lymphocyte count in six and eight weeks old birds in both summer and rainy seasons.

Glick (1967) and Siegel (1980, 1983) reported that stress might cause involution of lymphoid tissues, which reduced the number of circulating lymphocytes. Munck and Guyre (1991) opined that direct lysis or apoptosis and delay in maturation (Sapolsky, 1992) of lymphocytes due to the stress-induced increase in the circulating corticosteroids may further exacerbate the problem of reduced number of lymphocytes. Graczyk *et al.* (2003) reported that when broilers are subjected to stress, involution of lymphoid organs occurred due to continued activation of hypothalamo pituitary adrenal (HPA) axis and release of adrenal steroids, resulting in lymphocytopenia. Reddy (2003) also observed a significant lymphopenia in six and eight weeks old caged broiler chicks when the floor space allowance decreased from $734cm^2$/bird to $492cm^2$/bird.

The results of the present study were in support of the earlier reports. As per the observations of the experiment it may be concluded that stress due to overcrowding reduced the lymphocyte count and adversely affected the immune status of six and eight weeks old caged broilers in summer and rainy seasons. The results obtained in the mitogen induced lymphocyte blastogenic response also support the lymphopenia observed in the study.

Even though the dietary feed supplements improved the lymphocyte count in six and eight weeks old overcrowded broilers in summer, they failed to produce a similar beneficial effect in the rainy season. In this study vitamin C was found to be better than Protexin and virginiamycin to improve the immune status of the overcrowded birds.

The observations of the study indicated that age had a negative correlation with per cent of lymphocytes indicated by a reduced lymphocyte count from four to eight weeks in most of the groups under study.

Seasonal comparison of the average lymphocyte count in different groups of birds showed that heat stress reduced (numerically) the lymphocyte count in broiler chicks. However, the results of the study revealed that heat stress along with overcrowding stress produced a significant ($P \leq 0.05$) reduction in the lymphocyte count in birds. The antistress feed supplements used were very effective in reducing this combined effect of stress due to heat and overcrowding.

5.2.7 Heterophil / Lymphocyte (H/L) Ratio

The results of the study revealed that, H/L ratio recorded in the stressed control G-II group of birds was significantly ($P \leq 0.05$) high when compared to the birds in unstressed control G-I group. The antistress feed supplements reduced the H/L ratio in six and eight weeks old birds in the summer season. However, only vitamin C was found effective to reduce the H/L ratio in eight week old birds in rainy season.

From the results of the present experiment it is concluded that overcrowding (29 birds/m^2) produced a significant ($P \leq 0.05$) increase in H/L ratio in six and eight weeks old caged broilers in summer and rainy seasons.

In support with the observations of the present study Kuan *et al.* (1990) reported that increased stocking density produced stress indicated by higher H/L ratio from fourth week onwards. As per Emre *et al.* (1991), increase in population density from 10 and 14 chicks /m^2 to 18 and 22 chicks/m^2 increased the H/L ratio in 40 and 49 days old broiler chicks. However, in 1992, Cravener and associates found that seven week old birds reared at a floor space of 0.09 and 0.11m^2 / bird had significantly higher H/L ratio than those reared at 0.05 and 0.07m^2/bird. According to Patterson and Siegel (1998), cage density of 97cm^2/bird to 185.80cm^2/bird had no significant effect on H/L ratio in White Leghorn pullets, which disagreed with the results of the present study.

Gross and Siegel (1993) suggested that in birds H/L ratio was a good measure of long-term stress (hours or weeks). They observed that H/L ratio of about 0.2, 0.5, and 0.8 characterised low, optimum and high levels of stress respectively. The high value of H/L ratio (0.7 – 0.8) observed in the present study indicated high level of stress at a stocking density of 29 birds/m^2. Also the increased value of H/L ratio observed in the summer indicated that high temperature and humidity during the summer season is highly stressful to caged broilers. Supporting the findings of the present study Swenson and Reece (1996), Borges *et al.* (2004) and Mejo (2006) reported increased H/L ratio in birds subjected to heat stress.

On analysing the effect of the three antistress feed supplements it was seen that, vitamin C was found better than Protexin and virginiamycin (Table 5.5) and the Protexin was least efficient in reducing the H/L ratio in overcrowded caged broilers. An increased value of H/L ratio was noticed with age in all groups of birds, in both summer and rainy seasons, which indicated a positive correlation between age and H/L ratio.

5.3 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON BIOCHEMICAL PARAMETERS

5.3.1 Plasma Protein Profile

5.3.1.1 Plasma Total Proteins

Results of the experiment on the effect of overcrowding stress and antistress dietary agents on the concentration of plasma total proteins in six and eight weeks old broiler chicks in summer and rainy seasons revealed that the concentration of plasma total proteins in six and eight weeks old broiler chicks were not affected by stress applied. Seasons (summer and rainy) also had no significant influence on the level of plasma total proteins of birds. Different antistress agents used viz., Protexin, virginiamycin and vitamin C did not produce any significant change in the concentration of plasma total proteins in broiler chicken. However, the overcrowding stress applied in broiler chicken produced a non-significant reduction in the level of plasma total proteins in birds at both six and eight weeks of age in summer as well as rainy seasons.

Stress has been reported to increase protein catabolism (Brown *et al.*, 1958; Nagra and Meyer, 1963). Beard and Michell (1987) explained that during stress, body proteins are broken down and amino acids are shunted away from growth and are used by specific cells to synthesis critical proteins like acute phase proteins and antibodies which allow the bird to have a successful immune response and adaptation. One of the deleterious effects of stress responses in poultry is significant reduction in level of total proteins because of the increased corticosteroid secretion resulting in increased protein derived gluconeogenesis (Burger and Denver, 2002; Debut *et al.*, 2005). In the present study the reduction in the concentration of plasma total proteins due to the applied stress was not significant.

In support of the results of the present study, Reddy (2003) also observed a reduced (non significant) concentration of plasma total proteins in birds subjected to overcrowding stress (33% reduction in floor space). In the present study, 50 per cent reduction in the floor space did not significantly affect the concentration of plasma total proteins.

Seasonal influence on the concentration of plasma total proteins was also not significant. That is, cyclic temperature ranging from 22.83^{0}C to 32.68^{0}C did not significantly affect the concentration of plasma total proteins in broiler chicken. Zhou *et al.* (1998a) also did not observe any significant change in the plasma concentration of total proteins at temperatures of 7°, 20°, 25°, 30° and 35°C. However, Donkoh (1989) and Chunyan (1998) reported a reduced concentration of plasma total proteins in birds subjected to heat stress (30 and 35°C) that they attributed to the increased corticosterone concentration in heat stressed birds which caused a reduction in the amino acid transport from liver which in turn reduced the protein synthesis and reduced the concentration of plasma total proteins.

In the present study, even though no significant difference observed in the level of plasma total proteins of birds in summer and rainy seasons, the concentration of plasma total proteins of broiler chicken in all groups of birds reared in the summer season was numerically low when compared to that of the birds reared in the rainy season which may be due to the effect of heat stress. According to Nazifi *et al.* (2003) the total protein content was less affected in heat than in cold stress. Mejo (2006) reported a numerically lower protein concentration in cockerels subjected to heat shock (40±1.00°C) for five days when compared to the control birds, which support the results of the present study. Even though the protein concentration in stressed supplemented birds were not significantly higher than the stressed control G-II group of birds the numerical increase in the concentration of plasma total proteins in stressed supplemented groups suggested that the feed supplements were effective in preventing the protein catabolism during stress.

5.3.1.2 Plasma Albumin

The results regarding the influence of overcrowding stress and some antistress dietary supplements on the plasma albumin content of six and eight weeks old broiler chicks in summer and rainy seasons revealed that in the summer albumin concentration of six and eight weeks old broiler chicks were not influenced by overcrowding. The results of the present study, paralleled with the reports of Reddy (2003) that overcrowding stress (33% reduction in floor space) did not affect the albumin content of six and eight weeks old broiler chicks. He noticed an albumin level of 2.69g/dl in stressed birds at six week of age as against 2.57g/dl in unstressed control birds.

In the present study, during the rainy season, overcrowding resulted in increased albumin concentration in both six and eight weeks old birds. The antistress agents used were all effective in reducing the plasma albumin concentration significantly ($P \leq 0.05$) when compared to the stressed control birds. But virginiamycin failed to produce a significant reduction in the plasma albumin concentration in eight week old chicks in the rainy season (Table 6.2).

From the results it can be assumed that, in rainy season at a cycling ambient temperature ranging from 22.83^0C to 28.93^0C, stress due to overcrowding resulted in the increase of albumin concentration without any significant effect on concentration of plasma total proteins (Table 6.2 and 6.1). In summer cyclic ambient temperature ranging from 24.38^0C to 32.68^0C and the stress due to overcrowding did not affect the plasma albumin concentration. The feed supplements used in the trial were all effective in reducing the increase in albumin concentration produced due to overcrowding in rainy season.

In summer, a highly significant ($P \leq 0.01$) reduction in the plasma albumin content was noticed in six week old stressed control birds of G-II group when compared to those in the rainy season. However, since the control birds of G-I group showed no significant change in the albumin content between the summer and rainy seasons, it could not be concluded that cyclic temperature ranging from

22.83^0C to 32.68^0C had a significant effect on the plasma albumin concentration of broilers. A low level (nonsignificant) of plasma albumin concentration was noticed in all groups of birds in summer, when compared to their counterparts in the rainy season. The results were in agreement with the reports of Fengua *et al.* (1997) that the 350 days old laying hens exposed to an ambient temperature of 34.5°C for 10 to 14 days had a lower plasma albumin concentration. Later Mejo (2006) reported a numerically lower albumin concentration in cockerels subjected to heat shock (40±1.00°C) for five days when compared to the control birds, which paralleled the results of the present study. The significant reduction in albumin content was observed in stressed control G-II group of birds in summer than rainy season, which might be due to a combined effect of overcrowding and heat stress rather than the effect of heat stress alone.

5.3.1.3 Plasma Globulin

The results of the study revealed that overcrowding ($348cm^2$/bird) could significantly ($P\leq0.05$) lower plasma globulin concentration in six and eight weeks old birds in summer as well as rainy seasons. The three antistress agents (Protexin, virginiamycin and vitamin C) used in this trial were effective in reducing the adverse effects produced by the overcrowding on the plasma globulin concentration of the six week old birds under study. This beneficial effect of the dietary supplements on six week old birds was noticed in both summer and rainy seasons.

At eight week of age, virginiamycin was found to have no beneficial effect to improve the plasma globulin level of stressed birds in rainy season whereas it was found beneficial in summer. The probiotic (Protexin) as well as vitamin C improved the immune status of six and eight weeks old broiler chicks in both summer and rainy seasons.

As per the reports of Reddy (2003) stress due to overcrowding ($492cm^2$/bird) did not have any significant effect on plasma globulin level in six and eight weeks old birds. However, in the present study a significant ($P\leq0.05$)

reduction in the plasma globulin concentration was observed in overcrowded birds ($348cm^2$/bird). The diversity between the results of the present study and that of Reddy (2003) might be due to the high degree of overcrowding stress applied (50% reduction in floor space) in this study when compared to the low level of overcrowding (33% reduction in floor space) stress applied by Reddy (2003). Results of the present study, along with previous study by Reddy (2003), showed that even though, the stoking density of 22 birds/m^2 did not significantly affect the immune status of birds (represented by low globulin level) a stocking density of 29 birds/m^2 produced an adverse effect on immune status of the six and eight weeks old broiler chicks. The antistress agents used in the study could overcome the adverse effects of overcrowding on the immune status.

In the present study, when the globulin level of birds in summer were compared with their counter parts in the rainy season, there was no significant variation noticed in globulin concentration which agree with the reports of Mejo (2006) that there was no significant variation in globulin concentration in the heat stressed cockerels when compared with the non heat stressed cockerels. From the results of the study it could be assumed that hot humid summer season had no significant effect on the plasma globulin concentration of broiler chicken. The age of the bird also did not affect the plasma globulin level of broilers.

5.3.2 Albumin Globulin Ratio (A:G Ratio)

As per the results obtained in the present study on the effect of overcrowding stress and antistress agents on the A:G value of six and eight weeks old broilers in summer and rainy seasons it was seen that, overcrowding ($348cm^2$/bird) resulted in a significant increase in the A:G value of both six and eight weeks old birds in the rainy season and eight week old birds in the summer season. The antistress dietary supplements could not produce a significant ($P \leq 0.05$) reduction in the A:G value of overcrowded birds at eighth week of age in summer and sixth week of age in rainy season. On supplementing the vitamin C at 0.03 per cent level for 28 days produced a significant reduction in the A:G value.

There was no significant change in the value of A:G ratio with age in all the groups, especially in unstressed control G-I group of birds. The significant ($P \leq 0.05$) difference in the value of A:G ratio observed in some groups may be the combined effects of age, stress, treatment and season rather than the effect of age alone.

The A:G value of birds in summer and rainy seasons were statistically similar. So it could be assumed that season had no influence on the value of A:G in broiler chicks at fourth, sixth or eighth week of age.

Fenghua *et al.* (1997) noticed a lowered A:G ratio in 350 days old laying hens exposed to 34.5°C for 10 to 14 days when compared to those exposed to 26°C. The diversity between the results of the present study and that of previous reports may be due to the reasons as, broiler chicks were used in the present study instead of the laying hens in the earlier work, the birds used in the study were of four, six and eight weeks of age whereas in the earlier report the birds used were of 50 weeks of age and the birds in this study (before start of trial) were exposed to a temperature varying from 24.38^0C to 32.68^0C in the summer season and 22.83^0C to 28.93^0C in the rainy season and the temperature to which the birds were exposed before the period of trial in the earlier work might be much lower (which was not specified in the report) than 35°C and sudden exposure to 35°C might have resulted in the low A:G ratio.

Reddy (2003) reported that A:G value was not significantly affected in six and eight weeks old broilers subjected to overcrowding stress induced by reducing the floor space by 33 per cent. Along with the results of previous study results of the present study indicated that even though the stress due to 33 per cent reduction in the floor space did not induce any significant change in the A:G ratio, stress due to 50 per cent reduction in floor space resulted in a significantly ($P \leq 0.05$) high

A:G ratio. Along with the reports of Reddy (2003) the results obtained in this study suggested that increased A:G value in birds could be taken as an indicator of severe and chronic stress.

5.3.3 Electophoretic Seperation of Plasma Total Proteins

The electrophoretic movements of plasma from control and treatment groups did not show appreciable difference either between groups or between seasons. The results of the study suggested the absence of stress proteins in the blood of overcrowded birds in both summer and rainy seasons. The absence of stress proteins in the blood of overcrowded birds might either be due to the lack of production of any stress proteins or lack of appearance of the produced stress proteins in the plasma, as the stress proteins in the cells will appear in the fluid tissue like blood or plasma only on severe cellular damage.

5.3.4 Plasma Lipid Profile

5.3.4.1 Total Lipids

The results of the study indicated that stress due to overcrowding (50% reduction in floor space) produced hyperlipidemic effect in six and eight weeks old broiler chicks in the summer. The Protexin and vitamin C was found to produce a significant ($P \leq 0.05$) reduction in the plasma lipid level in overcrowded birds. However, the Protexin failed to reduce the lipid level in stressed birds at eight week of age. In six week old birds during summer virginiamycin supplementation in overcrowded birds of G-IV group produced a plasma lipid level which was less (non significant) than the stressed control birds of G-II group but higher than the unstressed control birds of G-I group. There was no significant difference in the plasma lipid level between G-IV (overcrowded birds supplemented with virginiamycin) and G-II (stressed control) birds as well as G-IV and G-I (unstressed control) groups of birds (Table 7).

In the rainy season, the stressed control birds in G-II group had a significantly ($P \leq 0.05$) high total lipid concentration compared to the unstressed control G-I group of birds. Unlike the observations in the summer, in the rainy season the anti-stress agents used could not reduce the elevated lipid level produced by overcrowding stress in birds of both sixth and eighth weeks of age.

The significantly high ($P \leq 0.05$) plasma lipid concentration noticed in the stressed control birds of G-II group both at sixth and eighth weeks of age in summer and rainy seasons suggested that, stress due to overcrowding could result in an increased plasma lipid concentration irrespective of season. This observation of the present study agree closely with the reports of Cetin and Tuncel (1995) that stress symptoms indicated by increased amounts of total plasma lipid could occur in birds of six and eight weeks of age when housing density increased to 22 birds/m^2 or more.

There was no significant difference in the concentration of total plasma lipid between summer and rainy seasons except in vitamin C supplemented overcrowded birds of six and eight weeks of age. However, a numerically low plasma lipid value was observed in the six and eight weeks old broilers in the summer when compared to the rainy season.

Freeman (1978) observed hypolipidemia in newly hatched chicks exposed to 40°C for 60 min. In the present experiment the non-significant reduction in the lipid concentration noticed in six and eight weeks old birds of summer season were in agreement with the earlier reports of Freeman (1978). The lack of significant change may be due to the thermo-tolerance developed with the advancement of age. The temperature to which the birds were exposed in the early part of their life might have also contributed to the thermo-tolerance.

A uniform increase in the total lipid concentration observed in the study from four to eight weeks indicated that during both phases of the trial, age (between 4 to 8 weeks) could influence the total plasma lipid concentration and the concentration of plasma lipid increased with age. That is, a positive correlation existed between age and total plasma lipid concentration. The increase in plasma lipid level was more in the summer season (represented by a significantly high plasma lipid concentration from 4 to 8 weeks as well as 6 to 8 weeks of age) than in the rainy season.

The results of the present study along with previous reports showed that, increase in plasma lipid concentration could be considered as a sign of overcrowding stress. Since the increased lipid concentration existed both at six and eight weeks, it can be assumed that the birds are not adapting to the stress as far as the plasma lipid concentration was considered. Of the three feed supplements (Protexin, virginiamycin and vitamin C) used in the trial, vitamin C was found to be effective in lowering the hyperlipidemic effect of overcrowding stress followed by Protexin and virginiamycin. The antistress agents were more effective to reduce the hyperlipidemic effect of overcrowding in summer than in rainy season (Table 7).

5.3.4.2 Triglycerides

The results recorded in the present study indicated that plasma triglyceride concentration was significantly ($P \leq 0.05$) high in eight week old stressed control G-II group of birds in summer whereas no such change was noticed in the rainy season. Even though the concentration of plasma total proteins was affected by the application of stress for a fortnight period, the changes in the triglyceride concentration occurred only on continued application of stress for 28 days. The dietary supplements used (Protexin, virginiamycin and vitamin C) were equally effective in reducing the increased triglyceride concentration resulted from heat and overcrowding ($348 cm^2$/bird) stress in eight week old birds in summer.

A cyclic temperature varying from 24.80^0C to 34.80^0C did not produce any significant change in the plasma triglyceride level of six week old broiler chicks. There was no significant increase in plasma triglyceride concentration in rainy season, either in six or in eight week old birds. But overcrowding produced a slight increase in the plasma triglyceride level in eight week old birds which was very insignificant. Since there was no increase in triglyceride concentration in six or eight weeks old birds in the rainy season, increased triglyceride value noticed in the summer season might be a combined effect of temperature and overcrowding rather than the effect of overcrowding stress alone. Similarly, a significantly ($P \leq 0.05$) high triglyceride value observed between the four and six weeks old birds in the rainy season might be attributed to the effect of overcrowding stress rather than the effect of age because in summer no such increase in triglyceride concentration was noticed between birds of different age groups.

As per the results obtained in the present study, summer as well as rainy seasons produced no significant effect on the plasma triglyceride value in six and eight weeks old broiler chicks.

Latour *et al.* (1996) reported an increased plasma triglyceride concentration on continuous infusion of ACTH at the rate of 8 IU/kg body weight/day and Puvadolpirod and Thaxton (2000a) also recorded increased plasma triglyceride concentration in broilers on continuous infusion of ACTH. Reddy (2003) did not notice any significant change in plasma triglyceride concentration in six and eight weeks old birds under overcrowding stress ($492 cm^2$/bird). According to Gursu *et al.* (2004), vitamin C supplementation in heat stressed broilers lowered serum triglyceride concentration.

The significantly increased plasma triglyceride concentration observed in eight week old birds in summer might be due to the stress produced by the combined effect of heat and overcrowding which might have resulted in the release of ACTH. The significantly decreased concentration noticed in the plasma triglyceride level in vitamin C supplemented overcrowded G-V group of birds are in support of earlier report of Gursu *et al.* (2004). The diversity between the result of the present study and that of Reddy (2003) might be due to the decreased amount of stress applied by Reddy (33% reduction in floor space) when compared to 50 per cent floor space reduction applied in the present experiment.

5.3.4.3 ***Total Cholesterol***

The results of the study on effect of the overcrowding stress (348cm^2/ bird) and effect of dietary supplements such as Protexin, virginiamycin and vitamin C on the plasma cholesterol concentration of six and eight weeks old broiler chicken in summer as well as rainy seasons revealed that, stress due to floor space reduction (50%) resulted in hypercholesterolemia in six and eight weeks old broiler chicks in both summer and rainy seasons.

The antistress agents used were effective in reducing the hypercholesterolemic effect of stress. Out of the three dietary supplements used, Protexin was found more effective than virginiamycin and vitamin C. Protexin could reduce hypercholesterolemia due to overcrowding stress both at sixth and eighth week of age as well as in both summer and rainy seasons. Moreover, the plasma cholesterol content in Protexin supplemented G-III group of birds were very near to the plasma cholesterol level of unstressed control G-I group of birds. Even though supplementation of virginiamycin reduced the cholesterol concentration in stressed birds, it failed to bring the cholesterol level similar to that of unstressed control G-I group of birds.

A uniformly significant ($P \leq 0.05$) increase in the cholesterol level was noticed with age in all groups of birds in summer as well as rainy seasons indicated that age (4 to 8 weeks) had a significant influence on the plasma cholesterol content of birds. Vitamin C could reduce hypercholesterolemia resulted with age (Table 7).

Rise in blood cholesterol was suggested to be one of the biochemical markers of stress response (Sayers *et al.*, 1945). As per the reports of Nagra and Meyer (1963), the corticosteroid injection increased the plasma cholesterol content in birds. Based on the earlier reports, hypercholesterolemia observed in the present study could be considered as a biological response to the overcrowding stress. Antistress dietary supplements used in the study were all effective in reducing the hypercholesterolemia (indicated by significant reduction in the increase of plasma cholesterol concentration) produced by the overcrowding stress.

The significant ($P \leq 0.05$) increase in the plasma cholesterol concentration noticed from four weeks to eight weeks period were in agreement with the earlier reports of Polonis (1982), who reported a significant increase in cholesterol concentration in birds from five to eight weeks of age. Donkoh (1989) noticed hypercholesterolemia in birds exposed to heat and cold stress whereas in the present study temperature ranging from 22.83^0C to 32.68^0C did not produce any significant effect on the plasma cholesterol concentration in four to eight weeks old broiler chicks vide Table 7.

Abdulrahim *et al.* (1996) opined that, *Lactobacillus acidophilus* reduced the serum cholesterol concentration in the blood by de-conjugating bile salts in the intestine thereby preventing them from acting as precursors in cholesterol synthesis. Mohan *et al.* (1996) evaluated the effect of probiotic at 75, 100 and 125mg/kg broiler diet up to eight weeks of age and noted that serum cholesterol was significantly reduced in probiotic fed groups. Birds, which received 100mg probiotic/kg diet, had the lowest cholesterol level (84.10mg/dl). As per Jin *et al.* (1998), supplementation of *Lactobacillus* cultures at 0.10 per cent level significantly ($P \leq 0.05$) reduced the serum cholesterol levels at 40 days of age. They concluded that the decrease might be due to cholesterol assimilation by *Lactobacillus* cells. Endo *et al.* (1999) observed that the serum cholesterol level was significantly ($P \leq 0.05$) reduced in cocks fed with a cholesterol-enriched diet and supplemented with probiotic (mixture of *Bacillus, Lactobacillus, Streptococcus, Clostridium, Saccharomyces* and *Candida).*

The effect of Protexin supplementation on the plasma cholesterol content of the overcrowded broilers was well in agreement with the earlier reports.

Results of the present study revealed that probiotic (Protexin) supplementation at the rate of 0.025 per cent in the broiler diet could reduce the cholesterol concentration in six and eight weeks old stressed broilers to that of unstressed birds in both summer as well as rainy seasons.

5.3.5 Plasma Glucose Concentration

Results of the study revealed that overcrowding ($348 cm^2$/bird) significantly ($P \leq 0.05$) increased plasma glucose concentration in both six and eight weeks old broilers in summer as well as rainy seasons. The dietary supplements were found effective in reducing the increased glucose concentration in six week old birds in both summer and rainy seasons. Even though the feed additives used in the experiment significantly ($P \leq 0.05$) lowered the hyperglycemic effect due to overcrowding in eight week old birds in rainy season, Protexin and virginiamycin failed to lower the glucose level of eight week old overcrowded birds in summer.

According to Cetin and Tuncil (1995), increasing the stocking density from 10 and 14 birds/m^2 to 22 birds/m^2 produced hyperglycemia in six week old broiler chicks. However, Reddy (2003) did not observe hyperglycemia in six and eight weeks old broiler chicks at a housing density of 22 birds/m^2 when compared to those at 14 birds/m^2. The results of the present study were in agreement with the results of Cetin and Tuncil (1995). Siegel (1971) and Chatterjee (1994) opined that stress increased circulating glucocorticoids, which have elevated the blood glucose concentration.

The significant ($P \leq 0.05$) increase in the plasma glucose concentration noticed between four and six weeks old birds in all the groups in both seasons of the present experiment proved the increase in the concentration of plasma glucose during the growing period.

The plasma glucose level of birds reared in summer and rainy seasons were statistically similar. This suggested that temperature varying from 22.83^0C

to 32.68^{0}C had no significant effect on plasma glucose concentration in four, six and eight weeks old birds. The combined effect of overcrowding and heat stress on plasma glucose concentration was also not significant than the effect of overcrowding alone. Observations by Deyhim *et al.* (1995) showed that exposing birds to heat stress did not have any significant effect on blood glucose level, which supported the results obtained in the present study.

The results of the present study suggested that increasing the housing density from 14 birds/m^2 to 29 birds/m^2 produced hyperglycemia in caged broiler chicks of six and eight weeks of age in both summer and rainy seasons. The antistress agents (Protexin, virginiamycin and vitamin C) used were effective in reducing hyperglycemic effect of stress in both summer as well as rainy seasons. The plasma glucose concentration of birds was not influenced by the seasons (summer and rainy seasons), but increased with growth during summer and rainy seasons.

5.4 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON HORMONAL PARAMETERS

5.4.1 Plasma Corticosterone

Analysis of the data on the effect of overcrowding stress and dietary antistress supplements on plasma corticosterone concentration of broiler chicken in summer and rainy seasons revealed that overcrowding for a fortnight period produced a significant ($P \leq 0.05$) increase in plasma corticosterone concentration of six week old birds in both summer and rainy seasons. However, on continued application of the same stress the increase in the concentration of corticosterone became nonsignificant. The antistress agents could reduce the corticosterone response to stress though the reduction was not significant. A significant reduction in the plasma corticosterone concentration was noticed with increase in age in all groups of birds reared in rainy season between ages of four to eight weeks. However, such a reduction was observed only in the unstressed control G-I group of birds in the summer season.

Siegel (1980) suggested that corticosteroid concentration in the blood could be used as a measure of environmental stress and physiological activity in chicken. Gould and Siegel (1985) recorded an increased serum corticosteroid concentration in broilers subjected to heat stress.

Siegel and Gould (1982) opined that prolonged and repeated exposure to heat would reduce corticosteroid response to heat stress. Hill (1983) opined that long-term response to stress involved release of ACTH from adenohypophysis, which stimulated adrenal cortex to produce corticosterone. On the other hand, according to Gross and Siegel (1983), H/L ratio was a better measure of long-term changes in the environment and changes in blood corticosterone concentration was a better measure of short-term stress.

El-Halawani *et al.* (1973) suggested that corticosterone concentration which increased shortly after exposure to stress returned to its initial concentration after two to three weeks depending on the intensity of stressor. Later it was supported by Buckland *et al.* (1974) and Edens and Siegel (1975) where they observed an increased circulating level of corticosterone concentration shortly after exposure to stressor. Beuving and Vonder (1978) observed an increased plasma corticosteroid level within minutes after injection of corticotropin into young or old laying hens or cockerels. This increase was more rapid in younger than in older birds.

Freeman *et al.* (1984) reported that transporting broilers for two to four hours and 220km resulted in higher plasma corticosterone concentration (4.5ng/ml against 1ng/ml in control birds) and the increase was more in summer than that in winter. Koelkebeck and Cain (1984) reported an increased corticosterone level in birds reared in floor pens at a stocking density of $0.094m^2$/bird when compared to that at a stocking density of $0.373m^2$/bird. By this study it was concluded that number of birds kept per cage would affect the physiological responses of birds to social stress.

Lagadic *et al.* (1990) by their experiment with caged layers in groups proved that the time taken to remove the blood sample did not influence the circulating corticosterone concentration. The study also revealed that, it was possible to take blood samples from birds kept in groups with out affecting the plasma corticosterone concentration in other birds from that group or in birds from other groups in nearby cages.

Latour *et al.* (1996) reported that continuous infusion of ACTH (8 IU/kg body weight/day) in chicken resulted in increased plasma corticosterone level which was further confirmed by Puvadolpirod and Thaxton (2000a; 2000c) when they observed an increased plasma corticosterone concentration in broilers, by two hours in response to continuous infusion of ACTH (16 IU/kg body weight/day for 7 days).

In the present study, the significantly ($P \leq 0.05$) higher plasma corticosterone concentration noticed in stressed control G-II group of birds could be the effect of overcrowding stress. The lack of a significant increase in the concentration of corticosterone in the plasma of stressed control G-II group of birds at eight week of age might be due to the adaptive response as reported by El-Halawani *et al.* (1973).

Kutlu and Forbes (1993) opined that ascorbic acid reduced the synthesis of corticosteroid hormone in birds and Sahin *et al.* (2002) observed a low concentration of ACTH in quails reared at 32°C and supplemented with vitamin C in the diet.

Satterlee *et al.* (1993) in a study in 18 days old Japanese quails subjected to stress for 12 to 21h with a reduction in floor space observed an increased corticosterone production. But supplementation of vitamin C at the rate of

1200ppm in drinking water failed to affect the adreno-cortical response to cooping combined with overcrowding. Present study revealed that ascorbic acid supplementation at the rate of 0.03 per cent (300ppm) in the diet resulted in a significant reduction in the corticosterone response to overcrowding stress. However, the supplemented vitamin C was not effective to bring down the corticosterone response to overcrowding stress combined with heat stress in hot humid summer months.

There was no significant difference in the plasma corticostrerone concentration between the birds reared in summer and rainy seasons. The lack of a significant increase in the plasma corticosterone concentration in the summer season might be due to the high environmental temperature to which the birds were exposed in the initial stages (from 1 to 4 weeks of age) leading to an adaptive response.

5.4.2 Plasma Cortisol

The study regarding the effect of overcrowding stress and effect of antistress agents on the cortisol concentration of overcrowded broilers revealed that in summer overcrowding for a fortnight period produced a significant ($P \leq 0.05$) increase in the cortisol concentration in six week old birds, whereas continued application of same stress produced only a numerical increase in the concentration of cortisol in eight week old stressed birds. However, in the rainy season stress due to overcrowding did not produce any significant change in the cortisol concentration of the six and eight weeks old broiler chicks.

Gill and Sharma (1992) opined that cortisol level in broiler chicken did not differ significantly between stocking densities (0.75 and 1.00sq.ft/bird). Reddy (2003) reported a significant increase in plasma cortisol level in broilers subjected to overcrowding stress (492cm^2/bird) when compared to the control birds (734cm^2/bird). He also observed a higher cortisol level (5.42ng/ml) in birds at sixth week of age than those at eighth week of age (5.00ng/ml) and he attributed it to increased resistance to stress with age.

The significant increase in the cortisol concentration observed in six week old birds agreed with the report of Reddy (2003) while the lack of significant difference in the concentration of cortisol noticed in the rainy season was in confirmation with the results of Gill and Sharma (1992).

In the present study, the significantly ($P \leq 0.05$) high concentration of cortisol observed in overcrowded G-II group of birds at sixth week of age might be the combined effect of overcrowding and heat stress rather than the effect of one stress alone. This was again supported by the non-significant increase in cortisol concentration in rainy season. The statistically similar value of cortisol concentration observed in eight weeks old birds in G-II group of phase-I might be the effect of adaptive response showed by the birds. Even though the increase was non-significant, the cortisol concentration in stressed control G-II group of birds were numerically higher than that of unstressed control G-I group of birds, which indicated the stress at higher stocking density.

On seasonal comparison, the birds in summer had a highly significant ($P \leq 0.01$) increase in the cortisol concentration when compared to their counterparts in the rainy season. This indicated increased stress at higher temperature (24.72 ± 0.46 to $33.16 \pm 1.17^{0}C$) in birds at all age groups (4, 6 and 8 weeks).

The antistress supplements were all effective in reducing the cortisol concentration of birds. However, Protexin and virginiamycin produce only a numerical reduction rather than a significant reduction in the cortisol concentration. Vitamin C produced a significant ($P \leq 0.05$) reduction in concentration of cortisol in overcrowded birds.

5.4.3 Plasma Triiodothyronine (T_3)

The study on the effect of overcrowding stress and antistress agents on the plasma T_3 concentration of six and eight weeks old broiler chicks in summer and rainy seasons revealed that overcrowding did not produce any significant effect on the plasma T_3 concentration in both summer and rainy seasons. The antistress supplementations did not have any significant effect on the plasma T_3 concentration of six and eight weeks old stressed birds, whereas, among the six week old chicks in the rainy season, the stressed birds in the Protexin supplemented G-III group and vitamin C supplemented G-V group had a plasma T_3 concentration significantly ($P \leq 0.05$) higher than the G-II group of birds. The birds of all age groups had a significantly low T_3 concentration in summer when compared to their counterparts in rainy season.

Heninger *et al.* (1960), Rogler and Parker (1978) and Bowen *et al.* (1984) reported a reduced T_3 and T_4 concentration in heat stressed chicken. Yahav (2000) observed a reduced plasma T_3 concentration when chicks were exposed to high ambient temperature. The significantly ($P \leq 0.05$) lower plasma T_3 concentration observed in the present experiment was in accordance with the earlier reports.

Results of the present study revealed that the stress due to overcrowding did not produce any significant change in the plasma T_3 concentration whereas the summer heat stress caused a significant ($P \leq 0.05$) reduction in the concentration of T_3. The antistress supplements failed to produce any significant change in the concentration of T_3 in heat stressed birds. The probiotic and vitamin C improved the T_3 concentration in overcrowded birds.

5.4.4 Plasma Thyroxine (T_4)

The results of the experiment on the effect of floor space reduction stress and the effect of some antistress feed supplements such as Protexin, virginiamycin and vitamin C on the concentration of plasma T_4 in six and eight weeks old broiler chicken in summer and rainy seasons indicated that overcrowding did not produce any significant change in the plasma T_4 concentration either in summer or in rainy seasons. The antistress feed supplementations had no significant effect on the plasma concentration of T_4. However, the plasma concentration of T_4 in birds of summer was significantly ($P \leq 0.05$) low when compared to those in the rainy season.

Significant ($P \leq 0.05$) increase in the plasma T_4 concentration noted in the summer might be due to the effect of fluctuating environmental temperature to which the birds were exposed. Analysis of climatic data during this period revealed a reduction in temperature and presence of summer rain (Table 4.1). It might also be the result of increased metabolism during the rapid growth period. The increased feed intake and weight gain observed during this period was also in support of the increased metabolism.

In summer, since the unstressed control birds of G-I group also showed a significant increase in plasma T_4 concentration between four and six weeks period, it could be assumed that a similar increase noticed in stressed control and treatment groups was not due to the effect of overcrowding stress. It was evident that stress due to floor space reduction did not affect the plasma concentration of T_4 in six and eight weeks old birds in both summer and rainy seasons.

The present experiment showed that age of the bird (between four and eight weeks) did not have any significant effect on the plasma T_4 concentration of birds.

Heninger *et al.* (1960), Rogler and Parker (1978) and Bowen *et al.* (1984) reported a reduced concentration of T_3 and T_4 in heat stressed chicken, while an increased level was noted by May *et al.* (1986) and Iqbal *et al.* (1990) due to heat stress in birds. Davison *et al.* (1985) opined that corticosterone produced during stress could affect circulating level of thyroid hormone too. Sinurat *et al.* (1987) reported that exposure of 21 days old chicks to 35°C for five hours per day increased T_4 and reduced T_3 level of plasma.

Buyse *et al.* (1991) concluded that clenbuterol supplementation (42mg/100 kg feed) for six weeks resulted in reduced T_4 concentration in female broiler chicks. They also reported that plasma T_4 level increased with age. In the present study the plasma concentration of overcrowded birds was not affected by the antibiotic (virginiamycin) supplementation in both summer and rainy seasons. Eventhough the plasma concentrations of corticosterone and cortisol were affected by the overcrowding stress applied, it did not produce any significant effect on the plasma concentration of T_3 and T_4 as reported by Davison *et al.* (1985).

The significantly ($P \leq 0.05$) low concentration of T_4 observed (during summer) in the study was in confirmation with the reports of Heninger *et al.* (1960), Rogler and Parker (1978) and Bowen *et al.* (1984).

Marcusen and Heninger (1976) reported that injection of vitamin C in rats resulted in a reduced thyroid function. Ladmacki *et al.* (1997) observed a significant reduction in the plasma T_4 levels at five and six weeks of age by dietary vitamin C supplementation (500ppm). However, they noticed a significantly higher T_4 concentration in the vitamin C supplemented birds at four weeks of age. As per the current study supplementing vitamin C did not produce a significant effect on the plasma T_4 concentration in overcrowded birds at both six and eight weeks of age as well as in both summer and rainy seasons.

5.5 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON LIVER FUNCTION

5.5.1 Plasma Enzyme Profile

5.5.1.1 Aspartate Amino Transferase (AST) Activity

The study on the effect of overcrowding stress and antistress feed supplements on the liver enzyme activity (AST) in six and eight weeks old broilers revealed that, the overcrowding ($348cm^2$/bird) produced a significant ($P \leq 0.05$) increase in the plasma AST activity in six week old broilers in both summer as well as in rainy seasons. In summer the significant ($P \leq 0.05$) AST activity was observed at sixth week, which persisted at eighth week of age. On the other hand in the rainy season the increase in AST activity at eighth week was not significant. The results indicated that cycling temperature ranging from 24.38 to 32.68 °C could produce liver and muscle damage (increased AST activity) in overcrowded birds. On the other hand, in rainy season, even though the overcrowding stress caused cellular damage (significant increase in AST) continuous exposure to same stress reduced the damaging effect.

Results of the comparison between summer and rainy seasons indicated that, the plasma AST value was not affected by a temperature range of 22.83^{0}C to 32.68 °C and humidity range of 88.25 per cent to 93.85 per cent. Therefore it can be assumed that the increased AST level observed was due to the effect of overcrowding stress.

All the three feed additives used in the study proved that they were equally effective in reducing the stress induced increase in the AST concentration in the overcrowded birds (348cm^2/bird) in both summer and rainy seasons.

Polonis (1982) observed a significant increase in AST activity between temperatures of 14°C to 26°C. However, in the present study no significant difference in AST activity was observed in birds between summer and rainy seasons. So it could be inferred that the extend of muscle and liver damage was similar in both summer and rainy seasons. The diversity between the results of the present study and that of previous study by Polonis (1982) might be due to the increased temperature (35.10 °C) to which the birds were exposed in the initial stage.

5.5.1.2 Alanine Amino Transferase (ALT) Activity

The results of the experiment on the effect of floor space reduction stress and the effect of some antistress agents (Protexin,virginiamycin and vitamin C) on the ALT activity of six and eight weeks old broiler chicken in summer and rainy seasons indicated that, the ALT activity of six and eight weeks old birds were not influenced by stress due to floor space reduction (50%) in both the seasons. No significant difference in the ALT activity was observed between the seasons. Both the floor space reduction applied as well as cyclic temperature ranging from 22.83°C to 32.68°C (Table 4.2 and 4.1) appeared to have no significant effect on the ALT activity in six and eight weeks old broilers. The effect of age on the plasma ALT activity of the birds was also not significant. However, a numerical increase in the ALT activity was noticed from four to eight weeks period in both summer and rainy seasons.

In summer the probiotic supplementation at the rate of 0.025 per cent in the diet produced a significant reduction ($P \leq 0.05$) in the ALT activity of the broiler chicken when compared to that of overcrowded birds fed with virginiamycin (0.02%). The result of the experiment revealed that Protexin and vitamin C could have a protective effect on liver (indicated by lower levels of ALT) in overcrowded birds at sixth and eighth week of age in the summer season. In the rainy season the virginiamycin was also effective in reducing the ALT level of stressed (overcrowded) birds. The high value of ALT noticed in G-IV (overcrowded birds supplemented with virginiamycin) group of birds in summer (April to May) can be due to the effect of initial high value of ALT observed in this group rather than the liver damage produced by virginiamycin. The value of ALT in G-IV group (40.58U/l) was very close to the value of control birds in G-I group (40.08U/l). It can be inferred that virginiamycin at 0.002 per cent level had no damaging effect on the liver in six and eight weeks old broiler chicken under stress due to the floor space reduction.

Polonis (1982) observed an increase in ALT activity between a temperature range of 14°C and 24°C. However, Fenghua *et al.* (1997) reported a significantly lower value of ALT at 34.5°C when compared to 26°C. Chunyan *et al.* (1998) also reported a reduced ALT concentration in fowls exposed to high ambient temperature. Results of the present study (cyclic temperature ranging from 22.83°C to 32.68°C appeared to have no significant effect on the ALT activity in six and eight weeks old broilers) was not in agreement with the earlier reports. The diversity between the results of present study and those previously mentioned studies might be either due to the differences in breed and age of broilers or the exposure of birds to high temperature in the initial period of life (1- 4 weeks of age) resulting in a thermal tolerance.

5.5.1.3 Lactate Dehydrogenase (LDH) Activity

The results of the investigation on the influence of overcrowding stress (50% floor space reduction) and effect of feed supplements as probiotic, antibiotic and vitamin C on the plasma LDH activity in six and eight weeks old broilers under overcrowding stress in summer and rainy seasons indicated that the 50 per cent floor space reduction in both summer and rainy seasons produced a significant ($P \leq 0.05$) increase in plasma LDH activity in six and eight weeks old broilers. The increase in LDH activity was observed both in summer and rainy seasons.

The plasma LDH activity is an indicator of muscle and liver damage, as the LDH activity will be increased in muscle and liver damage. The increased LDH activity observed in the present study suggested that 50 per cent reduction in floor space produced liver or muscle damage or damage of both. AST and LDH activity increase in both muscle as well as liver damage whereas increased ALT activity indicated liver damage.

In the current study, it was observed that the plasma AST and LDH activity were significantly ($P \leq 0.05$) higher in birds subjected to overcrowding stress (50% reduction in floor space) whereas the increase in ALT activity was meagre or even the value was less than that of control group (in summer at 6^{th} week of age). This significant increase in AST and LDH activity and lack of response in ALT activity clearly indicated that overcrowding stress applied in the experiment produced muscle damage than the liver damage. Appearance of the liver at the time of sacrifice (at the end of 8 weeks) also indicated that the liver was apparently normal.

Out of the three antistress agents used, the probiotic (Protexin) and vitamin C were found equally effective in reducing the muscle damage produced due to overcrowding stress. The beneficial effects of feed supplements especially Protexin and vitamin C was noticed in both summer as well as rainy seasons.

The results of seasonal comparison suggested that, variations in LDH activity of broilers was not significant between temperature range of 22.83°C to 32.68°C and humidity range of 64.55 per cent to 93.85 per cent. Chunyan *et al.* (1998) also did not observe any significant difference in the LDH activity in fowls exposed to high ambient temperature, which were in agreement with the results observed in the present study.

From the study, it was evident that, age of the bird had a significant ($P \leq 0.05$) influence on the plasma LDH activity. A positive co-relation existed between the age and LDH activity in broilers between four and eight weeks of age and the co-relation was noticed in both summer and rainy seasons.

5.5.1.4 Plasma Bilirubin Concentration

The plasma bilirubin concentration increased with liver damage and for the same reason, the concentration of bilirubin in the plasma is considered as an indicator of liver damage.

The result revealed that overcrowding stress did not produce much variation in the plasma bilirubin concentration. The dietary antistress agent supplementations also had no effect on the bilirubin concentration in the overcrowded birds in summer as well as rainy seasons. The fluctuating temperature ranging from 22.83°C to 32.68°C also had no effect on the bilirubin level. The result of the study clearly indicated that stress due to overcrowding (29 birds/m^2) and summer heat stress did not produce any significant liver damage. The statistically similar ALT concentration, which is another indicator of liver damage, also supported that overcrowding or seasons did not produce any liver damage in broiler chicken. These observations were again supported by the normal appearance of liver in all the birds at the time of sacrifice at eighth week.

5.6 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON THE IMMUNE PARAMETERS

5.6.1 Lymphoblastogenic Response

The proliferative response of lymphocyte to concanavalin and lipopolysaccharide (LPS) mitogen was suggested as a measure of lymphocyte proliferation (Toivanen and Toivanen, 1973, Hovi *et al.*, 1978; Haq *et al.*, 1996). In the present experiment it was seen that in both summer as well as rainy seasons the overcrowded birds in the group II (G-II) had a lymphoblastogenic response lower than that of control G-I group of birds. This reduced response was observed at both sixth and eighth week of age.

As per Blalock and Smith (1985) and Campos *et al.* (1991), the stress responses might be modified through the endocrine functions of the immune system. Bleecha *et al.* (1984) reported that the elevated glucocorticoids in stressed animals and birds were responsible to suppress the production of Interleukene-2 by lymphocytes, (an essential proliferative factor for lymphocytes) which thereby impairs the lymphocyte blastogenic response. The reduced lymphoblastogenic response noticed in the present study in the stressed control birds of G-II group might be due to the increased glucocorticoids produced by the overcrowding stress (348cm^2/bird).

Reddy (2003) also reported a reduced lymphoblastogenic response in broiler chicken subjected to overcrowding stress (492cm^2/bird). The results of the study were in agreement with the earlier reports.

Dietary supplementation of virginiamycin had an adverse effect on the lymphocyte proliferative response of the overcrowded birds. On the other hand Protexin and vitamin C improved the lymphoproliferation in response to mitogen in overcrowded broilers at sixth and eighth week as well as in summer and rainy seasons.

Vitamin C was found to be highly effective to improve the lymphoproliferative response, which is an indicator of immune response. Pardue *et al.* (1985) suggested that ascorbic acid supplementation (1000ppm) might alter endogenous synthesis and secretion of adrenal steroids and thereby limits the lymphotoxic effects associated with glucocorticoids and thus protect the immunological tissue from steroid insult.

The study revealed that overcrowding (348cm^2/bird) reduced the immune response (indicated by decreased lymphoproliferative response to mitogen) in six and eight weeks old broiler chicks in summer as well as in rainy seasons. Vitamin C can be effectively used to improve the immune response of overcrowded birds in both seasons.

Puthongsiriporn *et al.* (2001) reported that hens supplemented with a combination of 65 IU α - tocopherol and 1000ppm ascorbic acid had an additive effect in improving the *in vitro* lymphocyte proliferation in response to concanavalin-A and LPS mitogen when compared with α - tocopherol or ascorbic acid supplementation alone. Crowin and Shloss (1980) reported that vitamin E enhanced lymphoblastogenic response to mitogen by protecting the lymphocytes from lipid peroxidation by its antioxidant activity. Ascorbic acid had been demonstrated to enhance the antioxidant activity of vitamin E by decreasing the tocopheroxyl radical back to their active form of Vitamin E (Jacob 1995) or by sparing the available Vitamin E (Retsky and Frei, 1995).

The increased lymphoproliferative response observed in vitamin C supplemented G-V group of birds might be due to the antioxidant activity which protects the lymphocytes from lipid peroxidation and its ability to enhance the antioxidant activity of vitamin E by reducing the tocopheroxyl radical to vitamin E.

5.6.2 Concentration of Immunoglobulin G (IgG)

The results of the investigation on the effect of overcrowding stress and antistress agents on the IgG concentration of six and eight weeks old birds in summer and rainy seasons revealed that overcrowding stress adversely affected the IgG concentration of six and eight weeks old birds in both summer and rainy seasons (Fig.10).

Thaxton and Siegel (1973) reported that the antibody production could be impaired by stressors, which did not even evoke a rise in plasma corticosterone concentration. Later it was confirmed by Mashaly *et al.* (2004) who observed a reduced antibody production in laying hens exposed to constant heat and humidity (35^0C and 50% relative humidity).

The reduced IgG level observed in the overcrowded birds indicated a reduced immune function at high stocking densities, which in turn indicated stress at higher stocking densities.

In the study the antibiotic supplementation could not produce any improvement in the IgG level of overcrowded birds in both the seasons. The IgG concentration of six week old birds of virginiamycin supplemented G-IV group in the rainy season was even less than that of G-II group of birds which indicated that supplementing virginiamycin at the rate of 0.002 per cent in feed was not beneficial in improving the IgG concentration of overcrowded birds and can even adversely affect the immune status.

Even though the supplemented Protexin in summer failed to produce much increase in IgG level of overcrowded birds, in the rainy season the Protexin improved the IgG level of birds at both six and eight weeks of age. Pollmann *et al.* (1980) noticed elevated levels of serum globulin on oral inoculation of *Lactobacillus acidophilus* in germ free pigs.

From the present experiment it could be inferred that probiotic supplementation enhanced the immune status of overcrowded birds whereas it was ineffective in birds subjected to stress due to overcrowding and heat.

Supplementing vitamin C at the rate of 0.03 per cent improved the IgG concentration in six and eight weeks old birds in both summer and rainy seasons. However, it could not improve the IgG concentration of stressed birds in summer (indicated by low value of IgG in G-V group of birds in summer when compared to those in rainy season).

Pardue *et al.* (1985) reported that immunosuppression due to high environmental temperature (22.00 ± 0.80^0C) had been reduced by ascorbic acid supplementation at the rate of 1g/kg feed. It is suggested that ascorbic acid supplementation might alter endogenous synthesis and secretion of adrenal steroids thereby limiting the lymphotoxic effects associated with glucocorticoids or served to protect immunological tissue from steroid insult. Mc Corkle *et al.* (1980) suggested that ascorbic acid might modulate the B-lymphocytes but not T-lymphocytes. In the present study supplemented vitamin C could not improve the IgG concentration of stressed birds in summer to that of rainy season, which disagree with the earlier reports. This might be due to the very low (0.3g/kg) concentration of vitamin C used in the study when compared to1g/kg vitamin C used by Pardue *et al.* (1985).

While an increase in the concentration of IgG observed from four to eight weeks of age in all groups of birds in summer as well as rainy seasons, in G-II and G-IV group of birds the IgG level reduced from four to six weeks of age. This might be the effect of overcrowding. From this it could be confirmed that overcrowding reduced the immunity in birds and virginiamycin was not useful to improve the immunosuppression due to stress while Protexin and vitamin C could improve the immune status of overcrowded birds in both summer and rainy seasons.

5.7 EFFECT OF INDUCED STRESS AND ANTISTRESS AGENTS ON PRODUCTION PARAMETERS

5.7.1 Body Weight

The results of the study on the effect of overcrowding stress and antistress feed supplements such as Protexin, virginiamycin and vitamin C on the weekly body weight of broiler chicks revealed that overcrowding (348cm^2/bird) produced a significant ($P \leq 0.05$) decrease in the body weight of broiler chicks. The reduction in body weight was noticed both in summer and rainy seasons. The results of the present study revealed that overcrowding produced a negative effect on the body weight of broiler chicks in both summer and rainy seasons. The antistress agents used could not produce a significant improvement in the body weight of stressed broilers at all ages. However, vitamin C was found to be more effective than the other two antistress agents (indicated by increased body weight in G-V group when compared to other two groups). Vitamin C was more effective in improving the body weight in overcrowded birds in summer than in the rainy season. The body weight of four week old broilers in summer was found significantly ($P \leq 0.05$) less than those in rainy season. In five week old birds significantly ($P \leq 0.05$) reduced body weight was observed in G-II and G-IV group of birds. Among the six, seven and eight weeks old chicks even though the body weight was not significantly less in summer, the birds reared in summer months had numerically less body weight than those in rainy season. From these observations it could be assumed that the heat stress during the summer months adversely affect the body weight of broiler chicks and the adverse effect of heat stress was more in younger birds than in older birds.

Bolton *et al.* (1972) reported reduced body weight in broilers as the floor space allowance of birds decreased from $0.093m^2$/bird to $0.047m^2$/bird. However, Sulane and Ledayic (1976) observed no marked difference in the body weight of broilers in cage densities of 0.5 to 0.65sq.ft/bird. Cravener *et al.* (1992) reported that the body weight of broilers at 0.07, 0.09 and $0.11m^2$/bird was significantly high when compared to that of $0.05m^2$/bird indicated stress at higher stocking densities. Supporting the earlier report by Bolton *et al.* (1972) and Cravener *et al.* (1992) in the present study, it was seen that body weight of overcrowded, unsupplemented broilers at all age groups (from four to eight weeks age) were significantly ($P \leq 0.05$) lower when compared to the control G-I group of birds. In the current study, the reduced body weight observed in G-II group of birds indicated stress at stocking density of 29 birds/m^2 when compared to that of 14 birds/m^2.

Al-Batstan and Hussein (1999) observed that cyclic temperature reduced the body weight of broilers. According to Sinurat *et al.* (1987) exposure of broiler chicks of 21 days of age to high ambient temperature (35°C) for five hours per day for one month significantly ($P \leq 0.05$) reduced the body weight. They attributed this decrease in body weight to reduced feed intake at high ambient temperature. In the present study, the lower body weight observed in summer may be due to the reduced feed intake at high ambient temperature. The lack of significant difference in body weight at six, seven and eight weeks of age may be due to the high ambient temperature to which birds were exposed during the initial growth period, which resulted in thermal conditioning. As per Yahav and Mc Murtry (2001), short-term exposure to mild heat stress during the first week post hatch (36±1°C and 70 to 80% RH for 24h at 5 days of age) resulted in growth retardation followed by an immediate compensatory growth phase. In the present study, the high temperatures to which the birds exposed might have caused an initial reduction in growth (indicated by significantly lower body weight in four week old birds of summer), which was compensated in the later stages (Table 10).

Buresch *et al.* (1985) and Miles *et al.* (1985) suggested that incorporation of virginiamycin at the rate of 10 to 22mg/kg in feed improved the body weight in broilers. Proudfoot *et al.* (1990) did not observe any significant increase in the body weight in broilers supplemented with virginiamycin at a rate of 11mg/kg of diet. In the present experiment, the virginiamycin supplemented birds failed to produce a significant increase in the body weight. However, virginiamycin supplementation caused a numerical improvement in the body weight, which was in agreement with the reports of Proudfoot *et al.* (1990).

Mohan *et al.* (1996) observed that probiotic feeding (0.75mg/kg feed) significantly increased the body weight at fourth and sixth week of age but not at eighth week of age. Chithra *et al.* (2004) observed a highly significant increase in the body weight of broilers on dietary supplementation of probiotic at the rate of 0.3g per 60 chicks. In the present study, probiotic supplementation at the rate of 0.025 per cent in diet did not significantly improve the body weight in overcrowded broilers at five, six, seven and eight weeks in both summer and rainy seasons. However, it produced a numerical improvement in the body weight of overcrowded broilers.

Kafri and Cherry (1984) opined that ascorbic acid supplementation was beneficial during heat stress (32°C) but it produced poor performance at 23°C. According to Kassim and Norziha (1995), vitamin C supplementation in broilers reduced the effect of heat stress and improved the body weight. Sayed and Shoeib (1996) noticed that ascorbic acid supplementation in drinking water (0.5g/l) or in the diet (200g/kg diet) benefited the heat stressed broilers and improved the body weight by 6.8 per cent. As per the results of the present study vitamin C at the rate of 0.03 per cent in diet was found to be more beneficial in improving the body weight of overcrowded birds in summer than in rainy season, which was also in agreement with the earlier reports.

5.7.2 Body Weight Gain

Body weight gain of broilers in control and experimental groups revealed that even though the mean total body weight of broilers was significantly ($P \leq 0.05$) reduced by overcrowding (50% floor space reduction), the average weekly body weight gain was not significantly affected by overcrowding stress. However, in both summer and rainy seasons the gain in body weight at fifth week of age (1 week after application of stress) was significantly low in stressed control G-II group of birds. Vitamin C could produce a significant ($P \leq 0.05$) improvement in the body weight gain of birds. On continued application of the same stress the birds might have developed an adaptive response so that overcrowding stress did not produce any significant reduction in the body weight gain from sixth week onwards.

Kuan *et al.* (1990) observed that, when broilers were provided with a floor space of 0.095, 0.071, 0.051 and 0.048m^2/bird the growth rate was not affected by increasing the stocking density. However, after six weeks of age, the birds at lowest stocking densities had the highest average daily gain (39.2g) and those at highest stocking density had the lowest daily gain (32.20g). Supporting the earlier reports, in the present experiment, the weekly body weight gain of birds at lowest stocking density (696cm^2/bird) was higher when compared to that at highest stocking density (348cm^2/bird).

It was suggested that incorporation of virginiamycin at the rate of 10 to 22mg/kg in the feed improved the growth rate in broilers (Buresch *et al.*, 1985; Miles *et al.*, 1985). However, Proudfoot *et al.* (1990) did not observe any significant increase in the growth rate in broilers supplemented with virginiamycin at the rate of 11mg/kg of diet. March *et al.* (1978) observed a significantly increased weight gain in broilers supplemented with virginiamycin at a rate of 22ppm in the feed. In the current study, virginiamycin could produce only a numerical improvement in the body weight of broilers under overcrowding stress.

Ladukar *et al.* (2002) and Karaoglu and Durdag (2005) could not observe a better body weight gain on probiotic feeding. According to Borges *et al.* (2004), probiotic supplementation did not improve the body weight gain. As per the records of Mudalgi *et al.* (1993) feeding of *Lactobacillus acidophilus* or *Lactobacillus vulgaricus* (10×10^8cells/l of drinking water) to broiler chicks up to six week of age did not produce any significant change in the body weight gain. In the present study, the probiotic Protexin at a rate of 0.025 per cent in the broiler diet did not have any significant effect on the weekly body weight gain in broilers from fifth to eighth week of age. It produced only a numerical increase in the average weekly body weight gain from fifth to eighth week period.

Brown and Southern (1985) reported that ascorbic acid supplementation at one per cent level had no significant effect on body weight gain of broilers. However, as per the results of the present study, the weekly body weight gain of overcrowded ($348 cm^2$/bird) broilers was significantly ($P \leq 0.05$) improved by ascorbic acid supplementation at fifth, seventh and eighth week in summer and at fifth and eighth week in rainy season. The vitamin C was found more effective in summer than in rainy season to improve the body weight gain of overcrowded broilers, which was well in agreement with the reports of Pardue and Thaxton (1984) that growth inhibition produced by heat stress could be reduced by ascorbic acid supplementation.

5.7.3 Feed Intake

The results of the study revealed that overcrowding (29 birds/m^2) reduced the weekly feed intake of broiler chicks. The dietary feed supplements used could improve the feed intake of stressed birds in both summer and rainy seasons. However, the increased feed intake during rainy season in the Protexin supplemented G-III group of birds was meagre when compared to the stressed control G-II group of birds.

Shanawany (1988) reported a linear decrease in feed intake with densities over 20 birds/m^2. Kuan *et al.* (1990) also reported that when broilers were provided with a floor space of 0.095, 0.071, 0.051 and 0.048m^2/bird, with increasing stocking density the feed consumption decreased without affecting the growth rate. Experiment by Puvadolpirod and Thaxton (2000a) revealed that ACTH injection (8 IU/day, i/v for 7 consecutive days) resulted in reduced feed intake of broiler chicken.

In the present study the reduced feed intake observed in the stressed control G-II group of birds were in agreement with the earlier reports by Shanawany (1988), Kuan *et al.* (1990) and Puvadolpirod and Thaxton (2000c). The reduced feed intake might be due to the reduced metabolism in the overcrowded birds.

Deaton *et al.* (1978), Reece and Lott, (1983), Sinurat and Balnave, (1985) and Suk and Washburn, (1995) revealed that an increased environmental temperature reduced feed consumption and growth of meat type poultry. As per the reports of Sinurat *et al.* (1987), exposure of 21 days old broiler chicks to high ambient temperature (35°C) for five hours per day for one month significantly ($P \leq 0.05$) reduced the body weight and this was attributed to the reduced feed intake at high ambient temperature. Results of the present experiment also supported the earlier reports. Stress due to high temperature and humidity might have reduced the basal metabolic rate leading to a reduction in feed consumption. Stress due to overcrowding would also have resulted in a further reduction in the rate of metabolism, which again decreased the feed intake in stressed control G-II group of birds in summer.

Raynaud (1997) reported that antibiotic supplementation increased body metabolism, enhanced feed intake of the animals and birds and improved the weight gain. In the present experiment even though the virginiamycin supplemented G-IV group of birds showed a reduced feed intake at sixth week of age, the birds in G-IV group showed a better feed intake at fifth, seventh and eighth week of age. At the age of eighth week the birds in G-IV group showed the maximum feed intake of 1025.00g in summer.

Mahajan *et al.* (2000) suggested that supplementing the diet with probiotic under high environmental temperature improved feed consumption and feed efficiency. On the other hand as per the reports of Mudalgi *et al.* (1993), feeding *Lactobacillus acidophilus* or *Lactobacillus bulgaricus* ($10x10^8$cells per littre of drinking water) to broiler chicks up to six weeks of age did not produce any significant change in the body weight gain, feed intake or feed efficiency. Ladukar *et al.* (2002) could not observe any significant difference in feed intake and feed conversion ratio on probiotic supplementation. In the present experiment feeding of probiotic (Protexin) at the rate of 0.025 per cent in the feed produced improvement in the feed intake in both summer and rainy seasons.

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 stocking density and humidity by dissipating more heat, providing an opportunity for increased feed intake and greater layer performance. Sahota *et al.* (1994) studied the effect of ascorbic acid on silver black and White Leghorn chicks exposed to heat stress (39°C) and observed a better-feed intake and feed conversion efficiency. McKee and Harrison (1995) in their experiment on the effects of supplemental ascorbic acid on the performance of broiler chicken exposed to multiple concurrent stressors (beak trimming, coccidiosis and heat stress) found that ascorbic acid (150ppm) increased feed intake and improved the growth rate in stressed broilers. Kutlu and Forbes (1993) reported that reduced ascorbic acid concentration was associated with stress. However, according to Satterle *et al.* (1989) and Gross (1992) even though the ascorbic acid administration could protect against stress, it had no effect on performance parameters such as growth rate and feed intake.

Eventhough, a reduced feed intake noticed in stressed birds of G-V group at the age of sixth week, according to the results of the present study, ascorbic acid administered at the rate of 0.03 per cent improved the feed consumption in overcrowded birds and the effect was more in the summer season.

5.7.4 Cumulative Feed Efficiency (CFE)

The results of the study on the effect of overcrowding stress and antistress agents on the CFE of the broiler chicks indicated that the overcrowding improved the CFE of broilers from six week of age in both summer as well as rainy seasons. The dietary supplements used were all effective in further improving the feed utilization. Of the three dietary antistress feed supplements used, vitamin C was found to be better than the other two dietary supplements in summer season. The CFE was more in the summer than in the rainy season.

Siegel and Coles (1958) did not observe any significant difference in body weight and feed efficiency of broilers reared at various floor space levels ranging from 0.5 to 1.25sq. ft/bird. Whereas, Shanawany (1988) noticed a linear decrease in the feed intake with densities over 20 birds/m^2 with significant improvement in feed efficiency in the birds housed at high stocking densities.

Kuan *et al.* (1990) observed that when broilers were provided a floor space of 0.095, 0.071, 0.051 and 0.048m^2/bird, with increasing stocking density the feed consumption reduced and feed efficiency improved without affecting the growth rate. After the sixth week, birds at lowest stocking densities had the highest average daily gain (39.20g) and daily feed consumption (127.00g) and poorest feed efficiency (3.3g feed/g gain). Birds at the highest stocking density had the lowest average daily gain (32.20g) and feed consumption (90.60g).

In this experiment also, the feed consumption was reduced in overcrowded birds without much affect on the growth rate, which improved feed efficiency.

The observations in the present study were in accordance with the earlier reports. The diversity between the present study and that of previous reports by Siegel and Coles (1958) might be due to the more space allowance given by the early research workers (0.50 to1.25sq.ft/bird) compared to the floor space allotted (0.39sq. ft/bird) in the present study.

Al-Batshan and Hussein (1999) reported that the cyclic high temperature (26 to 34°C for 6h, 34±1°C for 12h and 34 to 26°C for 6h) reduced the body weight and feed efficiency. However, results of the present study was contradictory to the earlier report.

As per Yahav and Mc Murtry (2001), short-term exposure of chicks to mild heat stress during the first week post hatch (36±1°C and 70-80% RH for 24h at five days of age) resulted in the retardation of growth followed by an immediate compensatory growth phase. In the present study the high temperatures to which the birds were exposed during the early part of the life up to four week of age might have produced a compensatory growth resulting in a better feed efficiency in summer.

Yates and Schaible (1961) concluded that virginiamycin was quite effective in promoting the growth of chicks and poults. Buresch *et al.* (1985) recorded an increased feed efficiency in turkey poults supplemented with virginiamycin at the rate of 22mg/kg feed, when fed *ad libitum* as well as feed restricted by 25 per cent.

Miles *et al.* (1985) suggested that incorporation of virginiamycin at the rate of 10 to 22mg/kg in feed improved the growth rate and feed conversion ratio in broilers. However, Proudfoot *et al.* (1990) did not observe any significant increase in the growth rate and feed efficiency in broilers supplemented with virginiamycin at the rate of 11mg/kg of diet. As per the results of the present experiment supplementing the virginiamycin at the rate of 0.002 per cent in diet resulted in a better feed efficiency in broiler chicks under overcrowding stress in both summer and rainy seasons.

Mahajan *et al.* (2000) suggested that supplementing the diet with probiotic under high environmental temperature improved feed consumption and feed efficiency. Banday and Risam (2001) noted that supplementing probiotic at the rate of 75g per 100kg feed improved growth rate and feed efficiency. Chithra *et al.* (2004) obtained a highly significant increase in body weight and feed conversion in broilers on dietary supplementation of probiotics at the rate of 0.30g/60 chicks.

As per the reports of Mudalgi *et al.* (1993), feeding *Lactobacillus acidophilus* or *Lactobacillus bulgaricus* (10×10^8cells/l of drinking water) to broiler chicks up to six week of age did not produce any significant change in the body weight gain, feed intake or feed efficiency. Findings of Ergun *et al.* (2001) suggested that, in broilers supplementing probiotic with or without antibiotic had no effect on body weight gain and feed efficiency. In the present study too, it was seen that probiotic supplementation improved the feed efficiency in overcrowded as well as overcrowded heat stressed birds.

Freeman *et al.* (1983) observed no improvement in weight gain or survivability of birds when ascorbic acid was provided in the diet at a concentration of 1000mg/kg feed. Maus (1986) reported that the highest stocking density (18 and 23 birds/m^2) resulted in reduced weight gain, but was nearly compensated for the highest dose of vitamin C. According to Kassim and Norziha (1996), vitamin C supplementation in broilers reduced the effect of heat stress and improved the weight gain and feed efficiency.

The better feed efficiency in G-V group of birds observed in the study agreed with the earlier findings. The results of the present study along with the previous reports proved that vitamin C was beneficial in stressed birds than unstressed birds. Reinforcing this Pardue and Thaxton (1986) and Kutlu and

Forbes (1993) reported that environmental and pathological stressors are known to alter vitamin C synthesis and utilization in birds and administration of ascorbic acid had a protective effect against stress (Satterlee *et al.*, 1989; Gross, 1992).

5.7.5 Dressing Percentage

As per the results of the study, overcrowding produced no significant effect on the dressing percentage of birds at eighth week of age. The seasons also did not produce any significant change in the dressing percentage of birds. However, the dressing percentage was numerically higher in the summer than in the rainy season. This may be due to the reduced feathering in the hot and humid summer months. The feed additives used did not produce any significant effect on the dressing percentage of overcrowded birds in both summer and rainy seasons.

Takalikar *et al.* (1992), Baidya *et al.* (1994) and Mandal *et al.* (1994) reported that feeding probiotics did not produce any effect on the carcass yield. Samanta and Biswas (1997) inferred that *Lactobacillus bulgaricus, Lactobacillus acidophilus* or a combination of both at 10×10^{12} number of organisms/l of water in caged broilers upto six months had no effect on the dressed weight and eviscerated percentage as well as weight of giblet and spleen. Choudhury *et al.* (1998) observed no significant difference in dressing percentage, giblet yield and weight of liver, heart and gizzard on supplementing broilers with probiotic growth promoters at 0.05 per cent level. As per the results of the present study feeding antistress agents such as vitamin C did not have any effect on the carcass yield of overcrowded broilers.

5.7.6 Organ Weight

As per the results obtained in the study on the effect of overcrowding stress and antistress agents in the organ weights of overcrowded birds at eighth week of age in summer and rainy seasons revealed that, overcrowding produced no significant effect on organ weights in summer as well as rainy seasons. Fat percentage of the birds was also not affected by overcrowding.

As per the results of the study no organs were affected by the applied stress. Seasons also did not produce any effect on any of the organs in unstressed, stressed as well as stressed and supplemented birds. Fat percentage of eight week old broilers was also not affected by the overcrowding stress. Summer and rainy seasons were found to have no effect on the fat per cent of eight week old caged broilers.

No previous reports on the effect of stress on organ weights and fat content are available for comparing the results obtained in the study.

In the present study the Protexin did not produce any effect on the organ weights or on the fat percentage in summer and rainy seasons. Mohan *et al.* (1996) did not observe any significant difference in the weight of internal organs in probiotic-supplemented group when compared to the unsupplemented controls. Samanta and Biswas (1997) inferred that *Lactobacillus bulgaricus, Lactobacillus acidophilus* or a combination of both at 10×10^{12} number of organisms/l of water in caged broilers up to six months had no effect on the weight of giblet and spleen. Choudhury *et al.* (1998) observed no significant difference in giblet yield and weight of liver, heart and gizzard on supplementing broilers with probiotic growth promoters at 0.05 per cent level.

Chiang and Hsieh (1995) noted that supplementing probiotic at the rate of 0.25 and 0.5g/kg diet up to six weeks produced no significant difference in the abdominal fat content. Singh and Panda (1995) observed that the probiotic fed animals had lower fat content and higher protein content when compared to non-supplemented birds. Findings of Ergun *et al.* (2001) suggested that, in broilers supplementation of probiotic with or without antibiotic had no effect on edible visceral organ weight. Results of the present study suggested that probiotic supplementation at the rate of 0.025 per cent in diet did not have any significant effect on the giblet weight and fat percent of overcrowded chicks.

5.7.7 Economics of Production

The analysis of cost benefit ratio in control (G-I & G-II) and treatment (G-III, G-IV and G-V) groups of birds revealed that, overcrowding produced a net economic loss to the farmer. However, supplementing the overcrowded birds with the antistress feed supplements enhanced the net profit per kilogram of live weight. Vitamin C was found better than the virginiamycin and Protexin. The vitamin C supplemented G-V group of birds had a higher gain of 43 paise/kg live weight when compared to birds of unstressed control G-I group and an extra gain of rupees1.46/kg live weight compared to birds in stressed control G-II group. The Protexin supplemented G-III group of birds and virginiamycin supplemented G-IV group of birds had an economic gain less than the unstressed control G-I group of birds. However, they had a better economic gain when compared to the stressed control G-II group of birds.

In the rainy season also the same trend was noticed with highest economic gain of Rs.2.37/kg live weight in vitamin C supplemented G-V group of birds and lowest gain of Rs.1.38/kg live weight in stressed control G-II group of birds. Vitamin C supplemented G-V group of birds had a higher gain of 11 paise/kg live weight when compared to birds of unstressed control G-I group and an extra gain of 99 paise/kg live weight compared to birds in stressed control G-II group. The economy of production was better in summer than in rainy season in both control and treatment groups of birds. Vitamin C was found more effective to improve the economy of production in overcrowded and heat stressed birds than in birds under overcrowding stress alone.

Quinones *et al.* (1987) reported reduced growth rate in caged broilers when stocking density was increased. Kuan *et al.* (1990) concluded that stress associated with overcrowding reduced the performance in broilers. In the present study also, high stocking density of 348cm^2/bird produced poor performance leading to economic loss/kg live weight.

Dash *et al.* (1992) and Singh *et al.* (2000) revealed that antibiotic supplementation improved the growth rate in broilers. On calculation of economy of production per bird, a better gain was observed in virginiamycin fed group of birds when compared to other antibiotics fed groups (Singh *et al.*, 2000). Takalikar *et al.* (1992) and Prasad and Sen (1993) reported that the cost of feed per kilogram of weight gain was higher in probiotic-supplemented group (0.021 and 0.5g/kg feed). Banday and Risam (2001) noted that supplementing probiotic at the rate of 75g per 100kg feed improved growth rate, feed efficiency, carcass yield and profitability. The results of the study were in confirmation with the earlier reports.

From the results of the study it was concluded that the haematological parameters like count of heterophils, lymphocytes, basophils and H/L ratio were significantly affected by applying the stress of 50 per cent floor space reduction. Supplementation of virginiamycin at the rate of 0.025 per cent as antistress agent adversely affected the haematology of stressed birds. Changes were noticed in the biochemical parameters such as concentration of plasma globulin, total lipids, total cholesterol and glucose on inducing the overcrowding stress. A significantly high activity of AST and LDH was observed in the stressed control group of birds. Production parameters like body weight gain, feed intake and feed efficiency were also affected by overcrowding. Stress hormones like corticosterone and cortisol were increased significantly during the sixth week of age (after application of stress for a fortnight period). The study also revealed that the stress due to overcrowding reduced the immune status of the broilers. The use of antistress supplementations could rectify most of the stress-associated deviations and these applications were seen more effective during summer. Among the antistress agents used vitamin C was found to be superior in alleviating the stress associated changes. Prevailing climatic conditions did not produce much effect on the various physiological and production parameters studied.

Summary

SUMMARY

A study was conducted to evaluate the effect of overcrowding stress, season and dietary supplementation of certain antistress agents viz. probiotics (Protexin), antibiotics (Stafac-20) and vitamin C on the physiological, biochemical, hormonal and production parameters of broiler chicken (Vencob strain). The study was conducted in two phases, Phase I – in summer season, Phase II – in rainy season. One hundred and twenty, day-old broiler chicks were procured from a commercial hatchery and reared in battery cages under standard managemental conditions. At fourth week of age, the birds were divided randomly into five groups with 12 birds in each group viz. G-I – unstressed control (provided with a floor space of 696cm^2/bird), G-II – stressed control (provided with a floor space of 348cm^2/bird), G-III – provided with a floor space of 348cm^2/bird and supplemented with Protexin at the rate of 0.025 per cent in the feed, G-IV – provided with a floor space of 348cm^2/bird and supplemented with Stafac-20 (20g virginiamycin per kilogram) at the rate of 0.002 per cent in the feed and G-V– provided with a floor space of 348cm^2/bird and supplemented with vitaminC at the rate of 0.03 per cent in the feed. Each group was replicated twice.

The production parameters such as body weight, body weight gain, feed consumption, and feed efficiency were analysed on weekly basis. The dressed weight and dressing percentage as well as weight of giblet and percentage of fat were also analysed in eight week old birds.

Blood samples were collected with suitable anticoagulants at fourth, sixth and eighth week of age. Haematological parameters such as total erythrocyte count (TEC), haemoglobin (Hb) concentration, volume of packed red blood cells (VPRC), total leukocyte count (TLC), erythrocyte indices and differential leukocyte count (DLC) were analysed. Plasma samples were separated immediately after blood collection and used for biochemical, immunological and hormonal parameters. The blood biochemical parameters such as plasma protein profile (concentration of plasma total proteins, albumin, globulin and albumin:

globulin (A:G ratio), plasma lipid profile (concentration of plasma total lipids, cholesterol, and triglycerides), plasma concentration of glucose, bilirubin and activity of certain plasma enzymes such as aspartate amino transferase (AST), alanine amino transferase (ALT), and lactate dehydrogenase (LDH) were also estimated. The immunological parameters as plasma concentration of Immunoglobulin (IgG), lymphocyte proliferation response and heterophil:lymphocyte(H:L) ratio were analysed. Electrophoretic study was conducted to analyse the presence of stress proteins. Plasma concentration of certain hormones such as cortisol, corticosterone, triiodothyronine (T_3) and thyroxine (T_4) were also estimated. The data were statistically analysed using appropriate tools.

Overcrowding stress did not produce any significant change in the values of TEC, concentration of Hb, values of VPRC, MCV, MCH, MCHC, TLC, plasma concentration of total proteins and ALT activity. The antistress supplements did not have a significant effect on the values of TEC, concentration of Hb, values of VPRC, MCV, MCH, MCHC, TLC, concentration of plasma total protein and plasma ALT activity. However, virginiamycin supplemented overcrowded birds of G-IV group showed a significantly lower value of TEC, value of VPRC, MCV when compared to vitamin C supplemented G-V group of birds in both summer and rainy seasons. Age and season produced no effect on the values of TEC and TLC of controls (unstressed and stressed) as well as treatment (G-III, G-IV and G-V) groups of birds. The value of VPRC was not affected by the overcrowding stress whereas, virginiamycin supplementation in overcrowded birds (G-IV) significantly ($P \leq 0.05$) reduced the value of VPRC. At the age of four weeks, the value of VPRC was significantly ($P \leq 0.01$) lower in birds reared in summer than their counterparts in the rainy season.

Eventhough overcrowding produced no significant change in the concentration of Hb in six and eight weeks old birds in summer and rainy seasons, significantly ($P \leq 0.05$) lower concentration of Hb was observed in the overcrowded birds supplemented with virginiamycin (G-IV) at the age of eight weeks during the rainy season. Age of the bird (between 4 to 8 weeks) had no effect on the Hb concentration. Compared to those in the rainy season the Hb concentration of birds reared in summer was low.

The value of MCV was not affected by the applied stress, whereas in six week old overcrowded, heat stressed birds virginiamycin supplementation adversely affected the value of MCV and the value was lower (numerically) in summer than rainy season. The age of the birds did not have any significant effect on the value of MCV. The values of MCH and MCHC were also not affected by the applied stress, age of the bird or seasons. Antistress agents did not produce any significant change in the values of MCH and MCHC both in summer as well as rainy seasons.

Effect of overcrowding stress on the DLC showed that except the monocyte count the percentage of all other leukocytes were significantly ($P \leq 0.05$) affected by the applied stress. Season produced no significant effect on the monocyte count. The basophil count was increased by the overcrowding stress in four and six weeks old birds of both summer and rainy seasons. The antistress supplementations effectively reduced the increase in basophil count resulted by overcrowding stress. Dietary supplementations of Protexin and virginiamycin were found more effective than vitamin C in reducing the increased basophil count of stressed birds. The eosinophil count of six week old birds in stressed control G-II group during summer was increased significantly ($P \leq 0.05$) by the stress of overcrowding which could be effectively rectified by antistress supplementations.

The heterophil count was significantly ($P \leq 0.05$) increased by the stress in both six and eight week old birds of summer and rainy seasons. The antistress supplements could reduce the increase in heterophil count resulted from overcrowding in summer. Whereas, only vitamin C was found effective in the rainy season. Virginiamycin rectified the increase in heterophil count of six week old birds in rainy season but failed to have a positive effect on birds at eighth week. The heat stressed birds (in summer) of control G-I group showed a highly significant ($P \leq 0.01$) increase in heterophil count both in sixth and eighth week compared to their counter parts in the rainy season. The age of the bird showed a positive correlation with the heterophil count. The lymphocyte count of birds in stressed control G-II group was significantly ($P \leq 0.05$) lower compared to the birds of control G-I group. This reduction was noticed in birds at six and eight weeks as well as in summer and rainy seasons. The antistress supplements were effective in improving the lymphocyte count of overcrowded, heat stressed birds. However, supplements failed to improve the lymphocyte count of eight week old overcrowded birds in the rainy season. The H/L ratio was also significantly ($P \leq 0.05$) increased by the stress applied. The antistress supplements were effective to reduce the increase in H/L ratio associated with overcrowding stress in summer. In the rainy season only vitamin C could reduce the increased H/L ratio significantly.

The concentration of plasma total proteins was not affected by the applied overcrowding stress. The antistress supplements had no significant effect on the total plasma protein concentration of overcrowded birds. Eventhough a significant ($P \leq 0.05$) increase in the concentration of plasma total proteins noticed between the age of four and six weeks in the rainy season, no such change was evident in the summer. Seasons also produced no significant effect on the plasma concentration of total proteins. In the rainy season, the albumin concentration of plasma increased significantly ($P \leq 0.05$) by overcrowding whereas, in summer the increase was non-significant. A significant increase in plasma albumin concentration was noticed between four and six week old birds of summer and rainy seasons. The stressed control G-II group of birds in summer had a plasma albumin concentration significantly ($P \leq 0.05$) lower than that of rainy season. The plasma globulin level was significantly ($P \leq 0.05$) decreased by overcrowding stress in six and eight weeks old birds during summer and rainy seasons. Eventhough, the antistress supplementations significantly ($P \leq 0.05$) improved the plasma globulin concentration in six week old overcrowded birds in both seasons, virginiamycin failed to enhance the globulin level of plasma in eight week old overcrowded birds. Age and seasons did not produce a significant effect on the plasma globulin concentration of unstressed and stressed birds. However, a significant reduction in the plasma globulin concentration was noticed in stressed control G-II group of birds in rainy season. The albumin: globulin ratio was increased by overcrowding in both six and eight weeks old birds in rainy season and eight week old birds in summer season. The antistress agents failed to produce a significant reduction in the A:G ratio in summer whereas, they produced a significantly ($P \leq 0.05$) reduced value of A:G ratio in six week old birds in rainy season. Among eight week old birds of rainy season, only vitamin C was found to reduce the A:G ratio significantly. A fluctuating tendancy in A:G ratio was observed with age. Seasons produced no significant effect in A:G ratio.

The applied stress of overcrowding produced a significant increase in the plasma concentration of total lipids at both six and eight weeks of age during summer and rainy seasons. The antistress agents supplemented in the diet did not produce any significant reduction in the level of plasma total lipids in stressed birds. However, they produced a non-significant reduction in the level of plasma total lipids to have a concentration of total lipids not significantly more than that of unstressed birds. The Protexin was effective to reduce the increased plasma lipid level resulted by overcrowding stress. A significant increase in plasma concentration of total lipids was observed between birds at four and six weeks of age in rainy season whereas, such increase was noticed only in G-II and G-IV group of birds in summer. The plasma total lipid concentration of birds in overcrowded virginiamycin supplemented G-IV group of birds increased with age in both seasons. Seasons did not have a significant effect on the plasma total lipid concentration of birds. The overcrowding increased the plasma triglyceride concentration only in eight weeks old birds during summer. The antistress agents as Protexin and vitamin C significantly reduced the higher plasma concentration of triglycerides observed in eight weeks old birds in summer. The plasma triglyceride level showed a fluctuating tendency between ages in both seasons. The seasons also had no significant effect on the concentration of plasma triglycerides. Plasma concentration of total cholesterol was significantly ($P \leq 0.05$) increased in stressed control birds of G-II group both at six and eight weeks in summer as well as rainy seasons. The dietary supplementation of antistress agents reduced the elevated cholesterol level resulted from overcrowding stress. However, they could not reduce the level to that of unstressed control G-I group of birds. In six week old stressed birds in rainy season Protexin only could reduce the plasma cholesterol concentration. Whereas, continued supplementation of the antistress agents (Protexin, virginiamycin and vitamin C) for four weeks period effectively reduced the cholesterol concentration in both seasons. In summer and rainy seasons significant ($P \leq 0.05$) increase in the cholesterol concentration was observed between four and six weeks old birds. No significant change in the plasma cholesterol concentration was evident between seasons.

The plasma glucose concentration was also increased by the overcrowding stress in both six and eight weeks old birds during summer and rainy seasons. Except in eight week old birds of summer season the dietary supplementations effectively reduced the plasma glucose concentration. In eight week old birds of summer, only vitamin C could reduce the plasma glucose level significantly. Protexin and virginiamycin produced only non-significant reduction in the plasma glucose concentration. However, in the rainy season all dietary supplementation of antistress agents were found equally effective in reducing the elevated glucose concentration as a result of overcrowding. Eventhough, a significant ($P \leq 0.05$) increase in plasma glucose level was noticed in birds of four and six weeks, the increase in concentration of plasma glucose of six and eight weeks was only non-significant. No significant difference in plasma glucose concentration was observed between the summer and rainy seasons.

The study revealed that overcrowding produced a significantly ($P \leq 0.05$) elevated plasma AST activity in both six and eight weeks old birds of summer and six weeks old birds of rainy season. The antistress agents could reduce the increased AST activity. A significant ($P \leq 0.05$) increase in the AST level was noticed between four and six weeks in summer and between four and six as well as six and eight weeks of age in the rainy season. Between summer and rainy seasons, there was no significant change in the AST activity. The plasma ALT activity which is an indicator of liver damage was not affected by the applied overcrowding stress. A significant ($P \leq 0.05$) increase in the ALT activity was observed during the rapid growth phase of four to six weeks in both unstressed and stressed controls and treatment groups of birds during summer and rainy seasons. No significant difference in the ALT activity was noticed between summer and rainy seasons. The results of the experiment showed that the plasma LDH activity was significantly ($P \leq 0.05$) increased by the overcrowding stress in both six and eight weeks old birds during summer and rainy seasons. All the antistress supplements except virginiamycin were equally effective in reducing the plasma LDH level. Virginiamycin produced only a non-significant reduction in the plasma LDH activity. No significant variation in the plasma LDH activity was noticed between seasons in both six and eight weeks old birds. In both seasons a significant ($P \leq 0.05$) increase in activity of plasma LDH was observed with the advancement of age. A significantly lower LDH value was observed in four week old birds in summer whereas, in six and eight weeks old birds, seasons did not produce any significant effect on the plasma LDH activity.

The study revealed that the plasma concentration of corticosterone in six week old birds of stressed control G-II group was significantly ($P \leq 0.05$) higher than that of control G-I group birds in both seasons. However, continued application of same stress produced an adaptive response so that only a non-significant increase in the corticosterone concentration was evident in stressed birds at eighth week of age. The antistress agents supplemented in the study failed to produce a significant reduction in the plasma corticosterone concentration of stressed birds. However, they produced a numerical decrease in the plasma corticosterone concentration. Seasons had no significant influence on the plasma corticosterone concentration. The summer heat stress produced a non-significant increase in the value of plasma corticosterone, which was higher in eight week old birds than in four and six weeks old birds. The plasma corticosterone concentration of birds reduced with age in both seasons. The cortisol concentration of plasma increased significantly ($P \leq 0.05$) by overcrowding for a fortnight period in both summer and rainy seasons. Continued application of the stress resulted in a numerical rather than significant increase in the concentration of plasma cortisol. No significant change in the concentration of plasma cortisol was noticed with advancement of age of the bird. Seasonal comparison of plasma cortisol concentration revealed a highly significant ($P \leq 0.01$) increase in the concentration of plasma cortisol in summer compared to their counterparts in the rainy season. All the antistress supplements were effective in reducing the concentration of plasma cortisol in the rainy season. On the other hand only vitamin C was effective in significantly reducing the plasma cortisol concentration of overcrowded heat stressed birds. The plasma concentration of T_3 and T_4 were not affected by the overcrowding stress in six and eight weeks old birds during summer as well as rainy seasons. However the value of both T_3 and T_4 were significantly lower in summer than in rainy season. Rather than a steady increase or decrease in the value, a fluctuating tendency was noticed with age in plasma concentration of both T_3 and T_4

Overcrowding produced a reduction in the lymphoproliferative response of broiler chicks at sixth and eighth week of age as well as in summer and rainy seasons. The feed supplements produced an improvement in the lymphocyte proliferation response of overcrowded birds. The vitamin C supplemented stressed birds of G-V group had a lymphocyte proliferative response higher than that of unstressed control G-I group of birds. The IgG concentrations of birds were also decreased by the applied stress of overcrowding in both summer and rainy seasons. The antistress feed supplements improved the plasma concentration of IgG in stressed birds at both sixth and eighth week in both summer and rainy seasons. The plasma IgG concentration of birds during summer was less than their counterparts in the rainy season. The electrophoretic separation of plasma proteins of different groups did not revealed any of the stress proteins in the blood plasma.

There was no significant difference in the body weight observed between the unstressed control, stressed control as well as stressed birds supplemented with the antistress agents at fifth and sixth week of age. However, the body weight of birds at seventh and eighth week was significantly ($P \leq 0.05$) lower in stressed control G-II group of birds. Of the three antistress supplements, vitamin C produced a significant increase in the body weight in seven week old birds. At the age of eighth week, both Protexin and vitamin C were found equally effective to improve the body weight. Unlike the observations in summer, in the rainy season the body weight of birds in stressed control G-II group was significantly ($P \leq 0.05$) lower at fifth, sixth, seventh and eighth week. The feed intake of stressed control G-II group of birds was less than that of other groups at fifth week of age. The feed intake of birds in summer season was lower when compared to their counterparts in the rainy season. The overcrowding produced a significant ($P \leq 0.05$) reduction in the body weight gain of birds at fifth and eighth week of age during summer and rainy seasons. However, at the age of sixth and seventh week the gain in body weight was not affected by the overcrowding. vitamin C supplementation produced a significant ($P \leq 0.05$) effect in improving the weight gain of overcrowded birds both at fifth and eighth week as well as during summer and rainy seasons. The overcrowding lowered the cumulative feed efficiency (CFE) value in broilers at fifth week of age. However, from sixth to eighth weeks of age the CFE was more in birds of G-II group than that of G-I group in both summer and rainy seasons. The overall mean value of CFE was also better in G-II group of birds than G-I group of birds. The antistress supplements further improved the CFE value in stressed birds from sixth week to eighth week in both seasons. Vitamin C could improve the CFE of five week old overcrowded birds. As far as the overall mean value of CFE was concerned the overcrowded birds supplemented with vitamin C had the highest CFE.

The dressed weight and dressing percentage of eight week old birds were also not affected by the stress of overcrowding. The antistress supplements did not have any significant effect on the dressed weight and dressing per cent of overcrowded birds. Seasons also produced no significant effect on the dressed weight and dressing percentage. However, the dressing percentage was more (numerically) in summer than that in rainy season. The weight of different organs (heart, liver, spleen and gizzard) and fat percentage were also not affected by overcrowding stress. The dietary supplementation of antistress agents did not produce any significant effect on the organ weights and fat percentage in both seasons. The summer and rainy seasons also had no significant influence on the organ weight and fat percentage of eight week old broiler chicks. On considering the economy of production, it was seen that the overcrowded birds of G-V group supplemented with vitamin C gave maximum profit and stressed control birds of G-II group produced the least profit. The birds in summer had a better cost: benefit ratio than those in rainy season.

The results of the present study revealed that the overcrowding stress adversely affected certain haematological parameters (percentage of heterophils, lymphocytes, basophils as well as H/L ratio) biochemical (plasma concentration of globulin, total lipids, cholesterol, glucose, AST, and LDH) and production parameters (body weight, body weight gain, and feed intake). The increase in stress hormone concentration as corticosterone and cortisol indicated that stress was inflicted at higher stocking densities. The increased H/L ratio and basophil count were indicators of chronic stress suggesting that overcrowding for long period as from fourth to eighth week period could be leading to chronic stress. Immunity of birds (as measured by the lymphoproliferative response and plasma concentration of IgG) was also reduced by the applied overcrowding stress and the reduction was more in overcrowded heat stressed birds of summer season than birds subjected to overcrowding stress alone (in rainy season). Eventhough, seasons produced a significant effect on some physiological parameters it did not produce much effect on the production parameters. On the other hand the supplemented dietary antistress agents could rectify most of the stress-associated deviations in physiological and biochemical parameters. Since, the variations in different blood parameters ultimately reflects alterations in the various systems of the body, including muscular system, the stress- associated changes of blood picture may imply alterations in the meat quality of broilers. Profound knowledge about the actual physiological changes and implications thereof should form the basis for evolving judicious strategies to augment or optimise animal production, in respect of (farmers') economy, (consumers') safety and (animals') welfare.

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"EFFECT OF SEASON AND ANTISTRESS AGENTS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF BROILER CHICKEN UNDER STRESS"

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ABSTRACT

The study was conducted with the objective of ascertaining the efficacy of certain antistress agents as probiotic with multiple strains of bacteria and yeast (Protexin), antibiotic as virginiamycin (Stafac-20) and vitamin C in ameliorating the overcrowding stress induced during summer and rainy seasons in respect of physiological, biochemical, hormonal, immunological and production parameters of broiler chicken. The experiment was carried out on one-month-old Vencob broiler chicks in two separate phases; phase-I during summer and phase II during rainy season. In each seasonal phase, the birds were divided into five groups with 12 birds in each group. Each group was replicated twice. The birds in different groups were G-I – unstressed control birds (provided with a floor space of 696 cm^2/bird), G-II – stressed control (floor space reduced by 50% to have a space allowance of 348 cm^2/bird), G-III – overcrowded birds supplemented with Protexin at the rate of 0.025 per cent in the diet, G-IV- overcrowded birds supplemented with virginiamycin at the rate of 0.002 per cent in the diet, and G-V- overcrowded birds supplemented with vitamin C at the rate of 0.03 per cent in the diet.

Blood samples were collected from six birds in each replicate using appropriate anticoagulants. Haematological parameters were estimated using the whole blood. Plasma samples were separated and used for estimating biochemical and hormonal parameters. Quantification of immunoglobulin G (IgG) and electrophoretic separation of plasma proteins were carried out. Heterophil:Lymphocyte (H:L) ratio and lymphoblastogenic response were also recorded. The data were analysed statistically using appropriate tools.

Values of total erythrocyte count (TEC), concentration of haemoglobin (Hb), volume of packed red blood cells (VPRC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC) monocyte percentage, concentration of plasma total proteins, plasma alanine amino transferase (ALT) activity, plasma concentration of triiodothyronine (T_3) and thyroxine (T_4), organ weight of different organs as heart, spleen, liver, gizzard, fat percentage and dressing percentage were not significantly affected by applied stress of overcrowding. The percentage of heterophils, lymphocytes, basophils, H/L ratio, plasma concentration of globulin, total lipids, total cholesterol, and glucose, activities of plasma enzymes such as aspartate amino transferase (AST), lactate dehydrogenase (LDH), body weight, body weight gain, feed intake, and feed efficiency were influenced by overcrowding stress in both summer as well as rainy seasons. Although the concentration of stress hormones (cortisol and corticosterone) increased significantly ($P \leq 0.05$) by overcrowding stress in six week old broilers, continued application of the same stress caused waning off the increase to merely insignificant level. The applied stress and summer heat deteriorated the immune status of the broilers whereas the supplemented feed additives normalised many of the stress-induced changes in the haematological, biochemical and production parameters. Out of the antistress feed supplements used vitamin C was found more efficacious and economical than Protexin and virginiamycin in repealing the stress induced alterations in broilers at six and eight weeks of age both in summer and rainy seasons. Virginiamycin supplementation adversely affected the haematological parameters.

Seasons significantly changed the concentration of Hb, VPRC, heterophil count, lymphocyte count, H/L ratio, plasma LDH activity, plasma concentration of T_3, T_4, and cortisol as well as body weight of broiler chicken. The values of heterophil count, H/L ratio, plasma concentration of LDH, and cortisol were higher in summer, whereas, lower values were observed for Hb concentration, VPRC, lymphocyte count, plasma concentration of T_3 and T_4 and body weight. The birds in summer gave a better economical gain than those in rainy season. Haematological parameters such as TEC, TLC, MCH, percentages of monocytes, basophils, biochemical parameters such as plasma concentration of glucose, total proteins, albumin, globulin, A:G ratio, plasma concentration of total lipids, triglycerides, total cholesterol, AST, and ALT, corticosterone and production parameters such as body weight gain, organ weight, body fat percentage and

dressing percentage were not significantly affected by seasons. Both overcrowding and summer heat stress reduced the feed intake of broilers.

The plasma concentration of albumin, total lipids, cholesterol, glucose, AST, ALT, and LDH were increased significantly with the increase in age (4-8 weeks period) in both seasons. Significant reductions in the plasma concentration of corticosterone and T_3 were noticed with the advancement of age. The values of VPRC, TLC, plasma concentration of globulin, A:G ratio, plasma concentration of triglycerides and cortisol, were not significantly affected by the age of the birds (4 to 8 weeks).

From the results of the study it could be inferred that stress of overcrowding adversely affected many of the physiological, biochemical and production parameters of six and eight week old broiler chicken. Antistress feed supplements such as Protexin, virginiamycin and vitamin C were effective to rectify most of the stress-associated changes, but not all. The vitamin C was found better and more economical than other feed additives as Protexin and virginiamycin. Dietary supplementations of antistress agents were more effective in overcrowded, heat stressed birds of summer season than their counter parts of rainy season.