EFFICACY OF PROBIOTIC AND ASCORBIC ACID IN ALLEVIATING SUMMER STRESS IN GROWING BROILER RABBITS

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DECLARATION

I hereby declare that this thesis, entitled **"EFFICACY OF PROBIOTIC AND ASCORBIC ACID IN ALLEVIATING SUMMER STRESS IN GROWING BROILER RABBITS"** 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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Introduction

1. INTRODUCTION

In India Domestic rabbit, *Oryctolagus cuniculus* is gaining market popularity as a meat animal. Only recently, the nutritional attributes of rabbit meat has been recognized and broiler rabbit rearing has become a promising enterprise in the present day. In developing countries such as India, where enormous meat shortages exist, the potential for rabbit production is greatest. Minimal investment and labor costs, easy handling, high growth and fecundity rate and its highly profitable products makes rabbit farming a source of subsidiary income to an average farmer.

At present rabbit meat has got a high degree of popularity as protein food. Rabbits produce white meat that is fine-grained, high in protein (21%), low in lipid (5%), highly palatable, and low in cholesterol (59mg/100g) (Combes, 2004). Back yard rabbit rearing is an emerging enterprise in Kerala. The most important topic in rabbit research is to improve the production taking into account the farmer requirements, animal welfare and habitat. It is well known that rabbits are sensitive to extreme environmental conditions, like high humidity (Marai *et al.* 1994a) and high temperature (Okab *et al.* 2008). Kerala being a coastal state has got a hot humid climate. The most obvious limitation on rabbit production in Kerala is their susceptibility to heat stress.

Numerous studies have been carried out on the effects of heat stress in the hot summer of Kerala and its alleviation in other animals. However no much work has been carried out in rabbits to evaluate the effects of stress. A temperature of 21°C is known as the "Comfort Zone" for rabbits (Marai *et al.* 1994). Relative humidity recommended for optimum performance of rabbits is 55± 10 per cent (Prasanna *et al.* 2004) and temperature humidity index values above 27.8 results in heat - induced physiological stress in rabbits (Marai *et al.* 2002). Poor weight gains, impaired feed

conversion, increased disease incidence, decreased fertility and reduced reproductive efficiency results as a consequence of heat stress all of which adversely affects the production economics (Yamani and Khalil, 1994).

Alleviation of heat stress may be achieved by ameliorating the environmental stress, reducing the animals' heat production and or helping the animals' to dissipate the heat load. This includes physical, physiological and nutritional techniques (Marai *et al.* 1994b). Supplementation with probiotics and ascorbic acid to alleviate heat stress has been tried in chicken and found to be successful (Karthiayini, 2007 and Chitra *et al.* 2008). Dietary supplementation of the probiotic, *Lactobacillus sp.* can improve average daily weight gain and feed conversion ratio in New Zealand White rabbits (Amber *et al.* 2004). *Lactobacillus casei* has got a protective role against a toxin producing strain of *Escherichia coli* and increase the secretion of immunoglobulin A (Ogawa *et al.* 2001). Ascorbic acid being an anti stress vitamin can improve the growth performance during stress (Sayed and Shoeib, 1996). Ascorbic acid appeared to stimulate the immune status in animals (Ansari *et al.* 1998). At higher temperatures it significantly decreased mortality rate in rabbits (Skiivanova *et. al.* 1998).

Although studies on stress is voluminous on perusal of literature, reports on the effects of stress due to season in rabbit production is scarce. Besides the research on the antistress effects of growth promoters is also scanty. As such considering the importance of stress responses and its impact on broiler rabbit production, the present research work was envisaged with the following objectives.

1) To assess the physiological response to stress in broiler rabbits

2) To study the growth performance of rabbits during summer by the supplementation of probiotic and ascorbic acid

3) Cost effectiveness of supplementing probiotic and ascorbic acid to rabbits.

Review of Literature

2. REVIEW OF LITERATURE

2.1 CLIMATE

India is located in the northern hemisphere 8° 4" and 37 6" north and longitude 68 ° 7" and 97 ° 25" east and the climate in this region is described as tropical monsoon type (Anon, 1975).

The season in India had been classified into winter (October to February), summer (March to June) and rainy (July to September) (ICAR 1977).

As per the study results of Kumar *et al.* (2000) in semi arid region, the period October to march was most suitable for rabbit production. Summer period (April to June) was extremely hot and dry (35°C to 46°C; Relative humidity 15 to 35 per cent) and was unsuitable for rabbit production.

Relative humidity recommended for optimum performance of rabbits was 55± 10 per cent. Macro and micro climatic environment had an influence on productivity of rabbits (Prasanna *et al.* 2004).

2.1.1 Temperature humidity index

New Zealand White rabbits exposed to mild (temperature (14.98°C) and Relative Humidity (60 per cent)) and hot (temperature (29.74°C) and Relative Humidity (84 per cent)) climatic periods recorded Temperature humidity index values of 58.9 and 84.3 respectively indicating an absence of heat stress during the mild climate and exposure of the animals to severe heat stress during the hot climate (Marai *et al.* 1999).

Marai *et al.* (2001) modified the formula of Temperature Humidity Index (THI) for rabbits and was calculated as THI=db °C−[(0.31−0.31RH)(db °C−14.4)] where db °C=dry bulb temperature in degrees Celsius and RH=relative humidity percentage per 100. The values obtained are then classified as follows: <27.8=absence of heat stress, 27.8–28.9=moderate heat stress, 28.9–30.0=severe heat stress and 30.0 and more=very severe heat stress.

Temperature Humidity Index values of 18.5 during the mild climate and 33.9 in the hot climate period indicated absence of heat stress in the mild climate and exposure of rabbits to very severe heat stress in the hot climate (Marai *et al.* 2004)

In hot and humid climate, a significant rise was recorded in hemoglobin concentration, heterophil: lymphocyte (H: L) ratio in rabbits reared for a period of five months from March to July in hot-dry to hot-humid conditions. Temperature Humidity Index (THI) was below 80 in March (hot dry) which increased to 96 during July (hot humid) (Bothra *et al.* 2005)

Average temperature–humidity index (THI) values of 19.8, 18.0, 23.7 and 25.7 observed during autumn, winter, spring and summer, respectively in the rabbitry in subtropical climate of Egypt, indicated the absence of heat stress during autumn and winter (less than 22.2) and exposure to severe (more than 23.3) and very severe heat stress (more than 25.6), during spring and summer, respectively (Marai *et al.* 2006)

According to Marai *et al.* (2007) Temperature humidity index values of 20.2 indicated absence of heat stress and and 30.1 indicated exposure of rabbits to very severe heat stress in the latter case.

The humid tropical climate of the Southwestern Nigeria produced a higher temperature-humidity index (33.82 in 90° oriented pens and 32.49 in pens skewed (45°)

to the direction of the north-east prevailing wind) that gave a high level of heat stress for the rabbits. (Ogunjimi *et al.* 2008).

2.2 EFFECT OF CLIMATIC STRESS ON GROWTH PERFORMANCE OF RABBITS

Suc *et al.* (1996) observed that the does in underground shelter in Vietnam where the ambient temperature on an average was 3.8 °C lower and humidity 4.75 per cent higher in comparison with cages, were eight per cent heavier after two months of study and gave birth to 39 per cent more offspring and weaned 60 per cent more than that in cages. Survival rate upto weaning of offspring was improved by 16 per cent.

The hot and humid summer of Egypt reduced all parameters of economic importance in rabbit production. Treatment of rabbits with shearing and/or chilled drinking water significantly improved productive and reproductive traits and reduced the effects of heat stress on thyroid, kidney and liver functions and thermoregulation. (Abdel-Samee, 1997)

Most of the growth performance traits studied in New Zealand White rabbits were inversely affected in summer. The decrease in each of the growth performance and profit analysis traits during summer was mainly due to the decline in dry matter intake. (Marai *et al.* 1999)

Exposure of growing, adult male and female rabbits to higher Temperature-Humidity Index (THI) units as severe heat stress during summer adversely affected their growth and reproductive traits and reduced the resistance to diseases. The drastic changes that occurred in rabbits' biological functions were depression in feed intake, feed efficiency and utilization, disturbances in metabolism of water, protein,

energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites. When exposed to THI 30 or more, rabbits could no longer regulate internal temperature and heat prostration had set in. (Marai *et al.* 2002)

Raffa (2004) opined that temperature above 32°C induced heat stress in rabbits. The greatest loss from heat stress occurred at a temperature of 35°C

Okab *et al.* (2008) studied that poor growth performance of rabbits under heat stress was a result of the decrease in feed intake. High environmental temperature stimulated the thermal receptors to transmit suppressive nerve impulses to the appetite center in the hypothalamus causing the decrease in feed consumption. This lead to less protein biosyntheses and less fat deposition, leading to lowered body gain

2.2.1 Body weight

In growing New Zealand White rabbits the exposure to hot humid summer (temperature- humidity index was 84 ± 2) resulted in significant decrease in daily body weight gain by 24 per cent (Abdel-Samee, 1955)

High summer temperature of 27 °C and humidity 74 per cent led to a significant reduction in daily live weight gain in young meat rabbits. The average feed intake was significantly higher in winter compared to summer (36.1 versus 29.5g) in New Zealand White rabbits reared under hot environmental conditions (Cheiricato *et al.* 1993)

From weaning until 40 weeks, the live weight of the rabbits reared at high temperature (30°C) was lower than those reared in conventional building, where the temperature varied from 15 °C to 25°C (Pla *et al.*1994)

Rabbits adapted to semi arid conditions by reducing their body weights and thereby minimizing their heat loads. Young rabbits of meat breeds born in spring had poor post weaning growth than born in autumn and winter season. (Gupta *et al.* 1995)

According to Kamra *et al.* (1996) *Lactobacillus casei* alone did not have any significant effect on the daily body weight gain of rabbits

Rabbits reared in summer showed a reduction in final body weight, daily weight gain, feed intake, final margin (financial return), weights of carcass and kidney fat compared to those reared in winter (Ayyat and Marai, 1997)

Temperature of housing had a profound influence on performance and mortality of rabbits. The average daily gain of rabbits housed in 6, 16 and 25 ^{o} C was 30.8, 36 and 27.3g respectively (Skiivanova *et al.* 1998)

New Zealand White rabbits aged 35 days showed a decline in the final live body weight and body weight gain (0-7 weeks) by 14.1 per cent and 21.4 per cent respectively in summer compared to winter (Marai *et al.* 1999)

Gulyani *et al.* (2000) reported that season affected the performance of rabbits as there was a decrease in body weight during summer and increase during winter in the semi arid region of Rajasthan in India.

In growing New Zealand White rabbits the final live body weight and daily weight gain were significantly lower in summer (Temperature humidity index value of 28.9 indicated severe summer stress) compared to the winter group maintained on same diet and regime. (Marai *et al.* 2001)

The reduction in live body weight and daily body weight gain due to heat-stress conditions was due to the negative effects of heat-stress on appetite and consequent decrease in feed consumption (Marai *et al.* 2004)

The subtropical hot conditions of Egypt induced significant decline in final live weight and daily weight gain in five week old New Zealand and Californian male weaned rabbits. (Marai *et al.* 2005)

Das *et al.* (2006) revealed that the range of air temperature in the rabbitry was 29.03°C to 37.80°C and range of relative humidity was 80.25 to 63.11 per cent. It was found that average daily gain, dry matter intake and feed conversion ratio were negatively correlated with both air temperature and relative humidity.

Heat stress caused significant decrease in the final body weight and daily body gain in New Zealand White and Californian male weaned rabbits reared in the subtropical environment of Egypt (Marai *et al.* 2008)

A high correlation between weight gain and thermal comfort level existed in rabbits. The regression equation between rabbit weight gain and temperature humidity index showed a strong relationship ($WG = -2.6256(THI)^2 + 173.46(THI) - 2787.2$) (Ogunjimi *et al.* 2008)

Okab *et al.* (2008) observed that body weight was affected by the season and marked decreases were observed during summer compared with that of spring.

2.2.2 Feed efficiency

The impairment in production and reproductive performances in rabbits due to heat stress resulted from disturbances in animals' normal physiological processes and reduction in feed intake. (Abdel-Samee, 1955).

As per Das and Naik (1991) feed efficiency was negatively correlated with both air temperature and relative humidity.

High summer temperature of 27°C and humidity 74 per cent led to a significant reduction in daily intake and feed efficiency in young meat rabbits (Chiericato *et al.* 1993).

Does maintained at 12 and 18°C showed similar feed intake during gestation and lactation, while does maintained at 28°C showed a decrease, especially during the first 21 days of gestation and from second to fifth weeks of lactation. A marked depression in feed intake was always found in the group reared at 30°C (Fernandez *et al.* 1994)

New Zealand White rabbits reared in hot summer of Egypt showed a decline in the daily feed intake of 2.9 per cent compared to winter. The feed efficiency and water intake were higher in summer than in winter by 23.6 per cent and 157.8 per cent respectively. (Marai *et al.* 1999)

In growing New Zealand White rabbits the feed intake and digestability coefficients of dry matter (7.9 per cent) and crude protein (8.1 per cent) were significantly lower for the summer group compared to the winter group maintained on same diet and regime Temperature humidity index value of 28.9 was observed in summer indicating severe stress (Marai *et al.* 2001)

Supplementation of *Lactobacilli* improved digestibility of dry matter, crude protein, ether extract and crude fiber in rabbits. Improved digestibility of crude fiber in Lactobacilli supplemented rabbits was due to increase in the gut cellulolytic bacterial population as a result to enhancing lactate utilization and moderating pH of the media (Amber *et al.* 2004)

The subtropical hot conditions of Egypt induced significant decline in feed intake in five week old New Zealand and Californian male weaned rabbits. (Marai *et al.* 2005)

Rabbits reared in the humid tropical climate of the Southwestern Nigeria showed a significantly higher feed efficiency in those pens where the Temperature Humidity Index showed a more comfortable value. (Ogunjimi *et al.* 2008)

Lowest feed intake was recorded during the hottest parts of the summer in hybrid Hyla rabbits when reared in summer in Italy (Bovera *et al.* 2008)

Heat stress caused significant decrease in daily feed intake of New Zealand White and Californian male weaned rabbits reared in the subtropical environment of Egypt (Marai *et al.* 2008)

2.3 EFFECT OF CLIMATIC STRESS ON PHYSIOLOGICAL RESPONSE OF RABBITS

2.3.1 Rectal temperature and Respiration rate

Shafie *et al.* (1970) studied that the diurnal variation in body temperature of rabbits varied within a very short range (0.2–0.3 °C).

Richards, (1976) stated that water loss by respiratory frequency increased with increases in ambient temperature above panting threshold.

Dissipation of heat through respiratory water vapour was decreased by the increase in ambient humidity (Lebas *et al.* 1986).

As most of the sweat glands in rabbits are not functional and perspiration is never great because of fur, the only controlled means of latent heat evacuation is by altering the breathing rate (Marai *et al.* 1991).

Finzi *et al.* (1994) reported that the average body temperature in rabbits goes up from morning till night, while environmental air temperature goes up from morning till noon then decreases at night, indicating that body temperature is not affected instantly by changes in air temperature during the day.

Rabbits use general body position, breathing rate and peripheral temperature, especially ears temperature, as three devices to modify heat loss. However, respiration and ear are the most important dissipation pathways. In rabbits between environmental temperature of 0 and 30°C, latent heat evacuation is controlled by altering the breathing rate (Marai *et al.* 1994a).

Marai *et al.* (1996) recorded a respiration rate of 108.5±1.4 in rabbits during hot summer in Egypt.

Respiration rate and rectal temperature were higher by 4.33 per cent and 0.86 per cent respectively in New Zealand White rabbits reared in summer compared to winter. (Marai *et al.* 1999)

The subtropical hot conditions of Egypt induced significant increase in respiration rate and rectal temperature in 5 week old New Zealand and Californian male weaned rabbits. (Marai *et al.* 2005)

Marai *et al.* (2007) observed that a high significant increase in thermoregulatory parameters (respiration rate) and rectal temperature resulted due to exposure of animals to severe heat stress.

The physiological characteristics like rectal temperature and respiration rate were higher for rabbits in those pens with higher Temperature humidity Index values in the humid tropical climate of the Southwestern Nigeria. A low correlation existed between rectal temperature and thermal comfort level in rabbits. The regression equation between rectal temperature (RT) and temperature humidity index (THI) showed a low relationship ($RT = -0.0362(THI)^2 + 2.6417(THI) - 8.6967$). While a strong correlation between respiration rate and thermal comfort level existed in *rabbits. The regression equation between respiration rate* (RR) and *temperature* humidity index (THI) showed a strong relationship ($RR = 0.5451(THI)^2 - 32.495$ (THI) + 521.52). (Ogunjimi *et al.* 2008)

2.3.2 Estimation of glucocorticoids in stress

In hares, a small portion of glucocorticoids was excreted via the faeces and measuring faecal cortisol metabolite could be used as a valuable tool for non-invasive monitoring of disturbances in hares (Teskey-Gerstl *et al.* 2000).

The peak of hormone secretion occurs towards the end of the dark period in primates and other diurnal animals, whereas in primarily nocturnal animals like most *rodents and cats, there was a peak toward the end of the light period.* Therefore it is important to sample glucocorticoids at the same time of day if repeated measurements are to be made on different days or if comparing different groups or populations of animals. In species with a relatively long gut passage time (e.g., hind-gut fermenters) it might be impossible to detect diurnal changes of circulating glucocorticoid levels in the feces. (Touma, and Palme, 2005).

2.3.2.1 Faecal cortisol

On intravenous administration of radioactive-labelled glucocorticoids (^{14}C-cortisol and ^{3}H-corticosterone) to hares, peak concentrations were observed in the first urinary sample following infusion (13 ± 6 h) and in the faeces with a delay of about 1 day (23 ± 7 h) (Tesky – Gerstl *et al.* 2000).

Out of several potential predictor variables investigated, minimum ambient temperature and snow were only factors exerting a significant effect on fecal glucocorticoid excretion (Huber *et al.* 2003).

Janicki *et al.* 2006 proposed that significant difference in faecal cortisol was observed in stress during mating season in brown hare.

A significant increase in faecal cortisol metabolite concentrations was measured in guinea pigs 8 hours after the injection of Adreno Cortico Tropic Hormone. Peak concentrations were observed 18 h after the injection (Bauer *et al.* 2008).

2.3.2.2 Serum Cortisol

Rabbits subjected to surgical stress via laparotomy showed an increase in mean serum cortisol from 116.6 to 461.9 nmol per l (Toft *et al.* 1993)

Adult male Syrian hamsters when subjected to a psychological stressor like social defeat (i.e., exposure to a dominant animal in that animal's home cage) that was either acute (i.e., a single exposure) or chronic (i.e., daily exposure across five days) showed a significant increase in the serum cortisol concentration when

compared to the control group. Serum cortisol values were determined in a single Radio Immuno Assay (Jasnow *et al.* 2001)

The basal cortisol level in rabbits, determined by means of radioimmunoassay, was equal to 5.31±0.87 micrograms per cent over a period of 10 to 2 p.m. The blood collection procedure (ear marginal vein dissection) increased the cortisol concentration 30 minutes after blood collection by 19.4 per cent, i.e. augmented its level up to 6.37±0.90 micrograms per cent (Morla *et al.* 1984)

Szeto *et al.* (2003) opined that Rabbits, maintained in a controlled laboratory colony, secreted both corticosterone and cortisol in a circadian rhythm that peaked in the afternoon and reached a lowest at 0600 h.. Although corticosterone peaked at 1800 h and cortisol at 1200 h, values of each hormone were not significantly different at those two time points. Within the limits of the sampling rate of this study, it appeared that both glucocorticoids reached maximal levels between 1200 and 1800h.

High serum cortisol concentration (mg/dl) of 19.33+0.88 was obtained in summer when compared to winter (8.67+0.67) in New Zealand white rabbits maintained under hot climate of Egypt. Serum cortisol was higher by 122.95 per cent in summer compared to winter. Temperature humidity index value of 84.3 during the summer, indicating exposure of the animals to severe heat stress during the hot climate (Marai *et al.*1999).

2.4 DISEASE INCIDENCE DURING SUMMER SEASON

In rabbit farming season had influence in the incidence of the disease with higher number of cases being noted in summer as amount of ammonia gas produced during summer is generally greater than winter (Patton, 1984).

The study by Devi *et al.* (1990) revealed that pasteurellosis and intestinal coccidiosis were the major cause of death in rabbits. Highest mortality due to pasteurellosis was seen during the period from March to June. Season wise mortality revealed that it was highest in dry season and lowest in rainy season.

Nandakumar (1995) reported that exotic breeds like New Zealand White and Soviet chinchilla had heavy preweaning mortality, high incidence of diseases, sub optimal growth and reproduction under humid climate of Kerala.

2.5 MORTALITY DURING SUMMER SEASON

The study by Devi *et al.* (1990) revealed that mortality was highest in dry season (58 per cent) of the year and lowest in rainy season.

Yamani *et al.* (1991) reported that in Egypt, the highest per centage of still birth and pre weaning mortality were found in spring (10.2 and 36.2 per cent respectively) then followed by that in summer (4.1 and 22.4 per cent respectively)

Preweaning mortality rate (per cent) was higher in summer (71.9±14.9) compared to winter (27.8±7.2) in growing New Zealand White rabbits. Temperature humidity index value of 28.9 was observed in summer indicating severe stress (Marai *et al.* 2001).

In female rabbits age at puberty, pre and postweaning mortality increased by exposure to heat stress.(Marai *et al.* 2002).

Bacar *et al.* (2004) conducted a study to characterize morbidity and mortality in rabbitry during summer. The results of the study indicated that the main cause of mortality was diarrhea.

Month wise mortality pattern of rabbits in the Kamakshi Panchayath of Idukki district, Kerala revealed that the per cent of mortality among small, medium and large rabbit units were 42.7, 25.05 and 19.7 per cent respectively in the month of March. There was reduction in the number of kits produced in the month of February and March (27.66 ± 1.28 and 32 ± 0.53 respectively) in the case of small farmers (Chitra *et al.* 2007).

High temperature during summer period in combination with feed restriction significantly increased mortality rate in hyla hybrid rabbits. Highest mortality was recorded during the hottest part of summer when the maximum temperature reached 34.7°C. A mortality rate of 13.93 per cent was recorded in rabbits fed *ad libitum* feed during summer (Bovera *et al.* 2008).

2.6 ASCORBIC ACID SUPPLEMENTATION

Pardue and Thaxton (1984) reported that supplementation of ascorbic acid significantly ameliorated the immunosuppression associated with exogenous cortisol in chickens.

Njoku (1986) observed an improved growth performance in broilers, when the diet was supplemented with 200mg ascorbic acid per kg feed. Heat induced growth inhibiton, mortality and immune suppression were all reduced by ascorbic acid supplementation.

Degkwitz (1987) reported that the serum cortisol concentration increased with a decline in vitamin C supplementation below 90mg per cent in guinea pigs.

Sayed and Shoeib (1996) observed that ascorbic acid supplementation in drinking water (0.5 gram per litre) or in the diet (200 gram per kilogram diet) benefited the heat stressed broiler and improved the body weight by 6.8 per cent.

Ascorbic acid appeared to stimulate humoral immunity and antibody synthesis particularly IgG and IgA and also activated the macrophages (Ansari *et al.* 1998).

Ascorbic acid supplementation at the rate of 30 mg per kg of body weight twice a week to Hyla rabbits 2000 kept at 25° C significantly decreased mortality rate but other zootechnical parameters were not significantly influenced. Digestibility of nutrients, slaughter parameters and meat quality were not significantly influenced by ascorbic acid addition. Also, ascorbic acid addition did not improve weight gains or feed conversion in rabbits housed at 6°C and 25°C respectively (Skïivanová *et al.*1998).

New Zealand White rabbits when exposed to a higher temperature of 42° C showed a reduction in the immune cell mediated function and a transient increase in cortisol level. A significant increase in the plasma Vitamin C was noticed at 0.5h of heat stress. There was significant increase in rectal temperature and decrease in feed intake as a result of heat stress. (Amici *et al.*2000).

Ascorbic acid is probably the most effective and least toxic antioxidant identified in mammalian system (Rakeshkumar *et al.* 2001).

Ascorbic acid had a role in improving the digestability of nutrients in rabbits (Yacout et al. 2002).

Significantly higher feed intake, body weight gain and feed conversion ratio was observed in broiler chicken supplemented with 300ppm ascorbic acid during hot season (Kadim *et al.* 2008).

Vitamin C at a level of 250 mg per kg feed gave superior performance in final live weight and daily weight gain better than the control in broilers under heat stress.

But addition of Vitamin C at higher doses (500 mg and 750 mg/kg) gave lower performance even when compared with the control (Sabah-Elkheir *et al.* 2008).

Dietary supplementation of 200 ppm of ascorbic acid significantly increased live weight gain and feed conversion ratio in New Zealand White rabbits (Selim *et al.* 2008).

Supplementation of Vitamin C to heat stressed pregnant does reduced the pronounced adverse effects of heat stress on most of the physiological parameters and production traits. (Shebl *et al.*2008).

Dietary supplementation of ascorbic acid did not affect body weight gain and feed conversion ratio but quadratically changed daily feed intake of broilers at 21- 42 and 0 - 42 days of age (Konka *et al.* 2009).

2.7 PROBIOTIC SUPPLEMENTATION

Probiotic, lacto- sacc reduced the incidence of diseases and mortality rate in rabbits. The probiotic modified the gut bacterial population and the main disease control effect was reduction in the incidence of enteritis compared to the control group (Abdel- Samee, 1955).

The ability of the *lactobacilli* to produce toxic metabolites such as lactic acid, hydrogen peroxide and bacteriocin had been suggested as being responsible for their ability to inhibit other bacteria (Juven *et al.* 1992).

Daily supplementation of lactic acid producing bacteria (*Lactobacillus casei and Lactobacillus acidophilus*) at the rate of 5X 10^8 cells per animal per day for 84 days in 6 week old New Zealand White rabbits did not give any significant difference in feed intake, body weight gain and feed conversion efficiency. The feeding trial was

carried out at high temperature (40-44° C) and relative humidity 60-90 per cent. (Kamra *et al.* 1996).

Daily administration of sterilized artificial milk supplemented with *L. casei* strain Shirota at a concentration of 10^8 CFU per ml to Japanese White rabbits from the day of birth decreased the severity of diarrhea and lowered STEC (Shiga toxin-producing *Escherichia coli)* colonization levels in the gastrointestinal tract. By day 7 after infection 77.3 per cent of the rabbits in the control group suffered from severe diarrhea whereas only 16.0 per cent of the rabbits in the *L. casei*-treated group showed severe diarrhea. *L. casei* did not delay the onset of diarrhea. Administration of *L. casei* increased levels of Immunoglobulin A against shiga toxin 1 and shiga toxin 2 and formalin-killed STEC cells in the colon approximately two-, four-, and threefold, respectively, compared with those of the untreated controls by day 7 after infection (Ogawa *et al.* 2001).

Supplementation of *Lactobacillus casei* at the rate of 10^8 colony-forming units per ml thrice daily for three weeks inhibited intestinal bacterial pathogen, Helicobacter pylori (Cats *et al.* 2003).

According to Oyetayo *et al.* (2003) *L. casei* from fresh cow milk, was able to reduce the toxicological and pathological consequences associated with enterotoxigenic *E. coli* in experimentally infected rats. Another finding was that *L. casei* had a better probiotic effect than *L. acidophilus* in terms of liver function improvement, anticholesterolaemic property, and protection of the gastrointestinal tract from infection.

Supplementation of *Yucca schidigera* extract (250 mg per kg) or with 0.5 gm/kg Lact-A-Bac (dried *Lactobacillus acidophilus* 0.8 billion CFU per g) from 5 to 13 weeks of age significantly affected growth performance. Average daily gain increased by 12.1 or 9.6 per cent for rabbits feed diets with yucca extract or probiotic

respectively, as compared to control diet. Feed conversion ratio was improved by using treatment diets with means of 3.62 for treated and 3.87 for control rabbits. (Amber *et al.* 2004).

The probiotic bacterium, *Lactobacillus casei* induced activation of the gut mucosal immune system by an increased IgA level (Galdeano and Perdigon, 2006)

Male, 8 weeks old albino rats of Wistar strain had no variation in the body weight when fed synthetic diet containing 20 per cent fresh soyabean oil and lyophilized probiotic culture (*Lactobacillus casei casei* 19) or synthetic diet containing 20 per cent oxidized soyabean oil and lyophilized probiotic culture (*Lactobacillus casei casei* 19) indicating that the type of diet did not have any affect on the body weight. It was also observed that antioxidant present in the culture of *L. casei* ssp *casei* had an antioxidative effect similar to that of vitamin E (Kapila *et al.* 2006).

Supplementation of probiotics had a beneficial effect due to reduced mortality in growing rabbits (Matusevicius *et al.* 2006).

2.8 ASCORBIC ACID AND PROBIOTIC SUPPLEMENTATION

As per the study of Abd Elhalim (2008) ascorbic acid and probiotic (Roemin W2®) addition in the diet significantly improved feed conversion efficiency in growing New Zealand whilte rabbits.

Supplementation of probiotic and ascorbic acid independently and simultaneously either in feed or drinking water significantly increased the total serum proteins and reduced the serum cholesterol in broiler chickens (Chitra *et al.* 2008).

Karthiayini (2007) reported that Vitamin C produced a significant reduction in serum cortisol concentration but probiotic supplementation could only produce a numerical reduction in the cortisol level during heat stress in chickens.

2.9 ECONOMICS

Poor weight gains, impaired feed conversion, increased disease incidence, decreased fertility and reduced reproductive efficiency resulted as a consequence of heat stress all of which adversely affected the production economics (Yamani and Khalil, 1994).

In comparison with winter a decline of 3.1 per cent, 21.4 per cent and 31.55 per cent was recorded respectively for feed cost, return from body gain and in the final margin during summer in New Zealand White rabbits. (Marai *et al.* 1999).

Economics of rabbit farming depends mainly on the nutrition, economic ration, good health and high reproductive efficiency (Kumar *et al.* 2001).

Economically rabbits give highest per centage of return to investment. A female rabbit through its progenies can produce upto 80 kg of meat per year ie, 2900 to 3000 per cent of her live weight (Risam *et al.* 2005).

At environmental temperatures of 32°C and higher, heat stress occured, leading to production losses in hyla hybrid rabbits (Bovera *et al.* 2008).

New Zealand White rabbits supplemented with probiotic, Roemin W2® at 0.5 or 1 g/L showed an improvement in the economic efficiency or relative economic efficiency. (Abd Elhalim, 2008).

Materials and Methods

3. MATERIALS AND METHODS

The resources and facilities available at the Department of Livestock Production Management, College of Veterinary and Animal Sciences Mannuthy, Thrissur and Krishi Vigyan Kendra and Radiotracer Laboratory, Kerala Agricultural University, Vellanikkara, Thrissur were utilized for the study.

3.1 LOCATION

The study was conducted at Krishi Vigyan Kendra (KVK) of Kerala Agricultural University, Vellanikkara located seven kilometers east to Thrissur and is geographically situated at longitude 76°, 05" to 70°, 45" E, at latitude 10°, 20" to 10°, 56" N and at an altitude of 22.25 m above mean sea level. The location of the study is endowed with humid tropical climate.

3.2 PERIOD OF STUDY

As per the classification of Indian climate (ICAR, 1977), the summer season is from March to June. The climatic picture of Mannuthy (Thrissur district) described by Somanathan (1980) revealed that maximum temperature in March and April was above 32°C and that of May was above 30°C. Thus the period chosen for the present study was from March to May, covering three summer months.

3.3 EXPERIMENTAL ANIMALS

Twenty four weaned two month old New Zealand White rabbits (Figure 1) were randomly selected from Rabbit unit at Krishi Vigyan Kendra as uniformly as possible with respect to age and body weight and were utilized for the study.

3.4 DESIGN OF EXPERIMENT

3.4.1 Allocation of experimental animals to treatment groups

The animals were allotted randomly to the experimental units and each of the four treatments was replicated six times.

3.4.2 Treatments

Treatment – 1(T1)- Ascorbic acid (Merck, Mumbai) (Figure 2) at the rate of 200 mg per kg feed was given along with the basal diet

Treatment – 2(T2)- Probiotic, *Lactobacillus casei* (Unique Biotech, Hyderabad) (Figure 3) containing 10^6 colony forming units per g of feed was given along with the basal diet.

Treatment – 3(T3)- Probiotic and Ascorbic acid at the same rate as in T_1 and T_2 were given along with the basal diet.

Treatment – 4(T4)- Rabbits fed with basal diet alone (Package of Practices Recommendations, 2001, Kerala Agricultural University).

Fig. 1 - Experimental Animals

Fig. 2 - Feed Supplement
(*Lactobacillus casei*)

Fig. 3 - Feed Supplement
(Ascorbic acid)

3.5 MANAGEMENT OF EXPERIMENTAL ANIMALS

3.5.1 Housing

All the rabbits were housed individually with facilities for individual feeding and watering. The cages were cleaned daily in the morning. All the animals were reared under uniform managemental condition.

3.5.2 Feeding

All the animals were fed *ad libitum* fodder and water. The concentrate feed was given as per Package of Practices, Recommendations, 2001, Kerala Agricultural University. Animals were fed once in a day

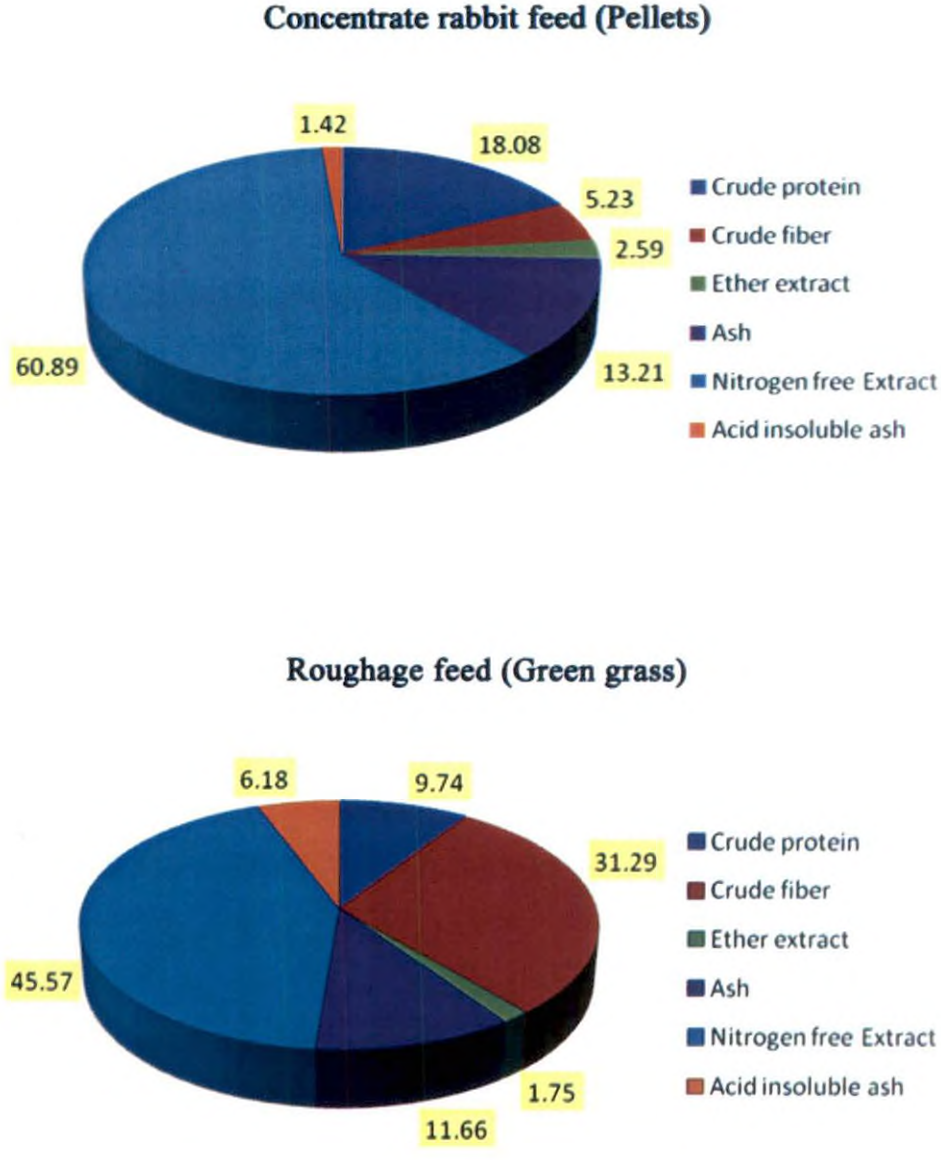
3.5.2.1 Proximate analysis of feed samples

Proximate composition namely moisture, crude protein, crude fibre, ether extract, ash, nitrogen free extract and acid insoluble ash of concentrate was (pellets) was estimated (A.O.A.C, 1990) and presented in Table 3.1 and Figure 4.

Table 3.1 Proximate composition of rabbit feed

Chemical Composition	Grass (% as DM)	Concentrate (% as DM)
Crude protein	9.74	18.08
Crude fiber	31.29	5.23
Ether extract	1.75	2.59
Ash	11.66	13.21
Nitrogen free Extract	45.57	60.89
Acid insoluble ash	6.18	1.42
Moisture (%)	**86.10**	**5.49**

Fig. 4 - Proximate composition of rabbit feed (percentage of dry matter)



3.6 ASSESMENT OF STRESS

3.6.1 Climatological data

The maximum and minimum temperature (°C) and relative humidity in the rabbitry were recorded daily with the help of Digital Hygrotherm (Sisedo) (percentage). The dry bulb temperature (°C) was recorded using dry bulb thermometer. The relative humidity (RH) and dry bulb temperature was recorded at 09.00 and 14.00 h daily. The meteorological data over a period from March 2009 to May 2009 were obtained from the meteorological observatory unit, Department of Metereology, College of Horticulture, KAU, Vellanikkara.

3.6.1.1 Temperature humidity index (THI)

The temperature–humidity index (THI) was estimated according to the formula as follows:

$$THI = db\ °C - [(0.31 - 0.31RH)(db\ °C - 14.4)]$$

where db °C = dry bulb temperature in degrees Celsius and RH = relative humidity percentage/100. The values obtained are then classified as follows: <27.8 = absence of heat stress, 27.8–28.9 = moderate heat stress, 28.9–30.0 = severe heat stress and 30.0 and more = very severe heat stress (Marai *et al.* 2001).

3.7 EFFECT OF CLIMATIC STRESS ON GROWTH PERFORMANCE OF NEW ZEALAND WHITE RABBITS

3.7.1 Body Weight

The body weight of rabbits were recorded at the start of the experiment and at regular weekly intervals using a standard weighing balance with 5 g accuracy and the average daily body weight gains were calculated (Biya, 2006).

3.7.2 Mean Daily Feed Intake

The rabbits were individually offered weight quantity of feed at 10.00h daily and residue was weighed next morning to record daily feed intake. Thereby average daily feed intake was calculated. (Kamra *et al.* 1996)

3.7.3 Feed Efficiency

Feed conversion efficiency was worked out on dry matter basis of feed (Banerjee, 1998)

3.8 EFFECT OF CLIMATIC STRESS ON PHYSIOLOGICAL RESPONSE OF NEW ZEALAND WHITE RABBITS

3.8.1 Respiration Rate

The respiration rate was recorded first among the physiological parameters. Reading was made from sufficient distance without disturbing the animal for two minutes and then average breaths per minute was recorded. It was expressed in cycles

per min. Respiration rate was recorded at weekly intervals in the different treatment groups (Marai *et al.* 1999).

3.8.2 Rectal Temperature

The rectal temperature of all the animals was recorded at weekly intervals using clinical thermometer (Marai *et al.*1999).

3.8.3 Estimation of serum and faecal cortisol by Radioimmuno assay

In order to assess the stress level in the different treatment groups serum and faecal cortisol levels were estimated. The method adopted for cortisol estimation was Radioimmuno assay (RIA). (Jasnow *et al.* 2001)

3.8.3.1 Collection and storage of serum samples

Blood samples were collected in the morning from the ear vein of the animal. Serum samples were separated on the day of collection and stored in deep freezer (-20°C) for cortisol assay. Samples were taken at monthly intervals.

3.8.3.2 Collection and storage of faecal samples

Faecal samples were collected at monthly intervals from all the animals in the early morning. They were kept in polythene pouches and stored at -20°C till extracted for RIA (Tesky- Gerstl *et al.* 2000)

3.8.3.3 Extraction of fecal cortisol for radioimmunoassay

The fecal samples stored at -20°C was crushed in the polythene pouch itself and thawed. Then 0.5 g per 50 g of homogenized wet feces was extracted with two ml distilled water and three ml methanol after vortexing the mixture for 30 min. It was then centrifuged at 2500 rpm for 15 minμ. A 0.5 ml aliquot of the supernatant was decanted and the feces residue in the centrifuge tube was again extracted with three ml methanol same as before. Again 0.5 ml of the supernatant was taken and mixed with aliquot already taken in the screw capped vial (Figure 5). The methanol extracts were stored at -20°C until RIA analysis (Palme *et al.* 1996)

3.8.3.4 Radioimmuno assay

Cortisol concentrations were measured using IM 1841 cortisol C T RIA kit (Immunotech, France) (Figure 6). From the six different cortisol standards 0, 19, 75, 219, 719, 1900 (nM) supplied with the kit, 50 μl each were incubated with 500 μl of I^{125} labelled cortisol tracer reagent in six anti cortisol monoclonal antibody coated tubes (rabbit anti-cortisol serum). Similarly, 50 μl of the methanol extract of fecal sample or 50 μl of the serum sample was incubated with tracer reagent in antibody-coated tubes. Control tube was incubated after adding 50 μl of control and 500 μl of tracer. In the tube marked for total count 500 μl of tracer alone was taken. After incubation for an hour at 18-25°C with shaking at 400 rpm, the contents of the tubes except the tubes for total count were decanted. The counts per minute (cpm) bound for each tube were counted in 1480 WIZARD TM Automatic Gamma Counter for one minute with the window suitably adjusted for Iodine-125 (Figure 7). The counts per minute obtained for all the samples were divided by counts per minute for zero calibrator and the result was denoted as B/B0 in percentage. A semi logarithmic curve fit (spline mode) with B/B0 (%) on vertical axis and the cortisol concentration of the

Fig.5 - Faecal sample extraction

Fig. 6 - Cortisol RIA kits

Fig. 7 - Radio Immuno Assay (RIA)

calibrator on the horizontal axis (nM/l) was plotted. For each sample, B/B0 (%) on the vertical axis was located and the corresponding cortisol concentration on the horizontal axis was determined. The concentrations in nM per l were converted to ng per ml by multiplying the values by 0.362. The results were expressed in µg per dl.

3.9 DISEASE INCIDENCE AND MORTALITY

Incidence of any diseases or mortality was recorded during the experimental period.

4.0 COST EFFECTIVENESS OF SUPPLEMENTING PROBIOTIC AND ASCORBIC ACID

The cost of production on feed basis of the four treatments were determined (Biya, 2006).

4.1 STATISTICAL ANALYSIS

Data collected on various parameters were statistically analyzed as per the method of Snedecor and Cochran (1994) by employing one way analysis of variance (ANOVA)

Results

4. RESULTS

4.1 ASSESMENT OF STRESS

4.1.1 Macroclimatic changes

The mean of the macro climatic variables such as maximum and minimum temperature, relative humidity at morning and after noon are presented in Table 4.1. The mean maximum temperature was highest in March and lowest in May. The mean minimum temperature was almost same in March and April but it was comparatively low in May. The relative humidity was highest in May both in the morning and afternoon.

The mean maximum temperature in the three summer months were 34.62±0.06°C, 33.61±0.07°C and 30.61±0.04°C respectively and the mean minimum temperature were 25.03±.04°C, 25.17±.05°C and 23.90±.03°C. The mean humidity (morning) in the three months were 89.19±0.11%, 86.93±0.17% and 93.6±0.10% and the mean humidity (afternoon) were 58.06±0.28%, 58.93±0.31% and 71.20±0.28% respectively.

4.1.2 Micro climatic changes

The mean of the microclimatic variables such as mean monthly maximum temperature, minimum temperature, relative humidity (morning) and relative humidity (afternoon) are furnished in Table 4.1. The mean of weekly dry bulb temperature, weekly relative humidity and temperature humidity index (THI) values (weekly and monthly) have been presented in Tables 4.2, 4.3, 4.4 and 4.5 and Figures 8-11 respectively. During the experimental period the temperature humidity index (THI) values were high in the afternoon compared to the morning. The THI values were highest in April (28.50±0.22) and lowest in May (26.83±0.21).

Table 4.1 Environmental variables during the experimental period

Parameters	Months	Macroclimate	Microclimate
Maximum temperature(°C)	I	34.62±0.06	34.12±0.03
	II	33.61±0.07	33.20±0.03
	III	30.61±0.09	30.01±0.04
Minimum temperature(°C)	I	25.03±0.04	25.1±0.04
	II	25.17±0.05	25.6±0.02
	III	23.90±0.03	24.1±0.02
Relative humidity morning (%)	I	89.19±0.11	90.34±0.16
	II	86.93±0.17	88.56±0.11
	III	93.60±0.10	92.68±0.07
Relative humidity afternoon (%)	I	58.06±0.28	59.52±0.25
	II	58.93±0.31	61.75±0.12
	III	71.20±0.28	71.19±0.33

Non significant (P>0.05)
I- March, II-April, III-May

Table 4.2 Mean weekly dry bulb temperature (°C) in the rabbitry

Weeks	Dry bulb temperature (morning)	Dry bulb temperature (afternoon)
1	25.37±0.31	33.46±0.45
2	27.26±0.13	34.79±0.45
3	26.66±0.25	33.99±0.70
4	26.17±0.55	32.06±0.57
5	27.09±0.23	31.59±1.29
6	27.77±0.17	33.89±0.24
7	27.77±0.52	33.66±0.29
8	27.03±0.59	32.76±0.33
9	25.80±0.17	31.41±0.74
10	25.57±0.45	29.94±0.60
11	26.20±0.55	30.53±0.65
12	25.11±0.30	29.26±1.02
13	25.29±0.44	29.77±0.32

Table 4.3 Mean weekly Relative Humidity (percentage) in the rabbitry

Weeks	Relative Humidity per cent (morning)	Relative Humidity per cent(afternoon)
1	89.43±1.88	57.00±3.13
2	90.29±1.10	56.57±1.23
3	92.14±1.20	58.43±3.94
4	91.71±0.71	66.29±1.90
5	89.86±1.22	62.29±4.25
6	88.20±1.04	59.71±1.41
7	86.00±1.48	58.71±2.40
8	89.00±2.57	63.86±2.57
9	92.14±0.96	69.00±3.23
10	92.71±1.06	73.29±2.47
11	92.14±0.91	69.29±3.07
12	93.86±0.91	73.19±4.52
13	90.86±0.96	72.00±1.88

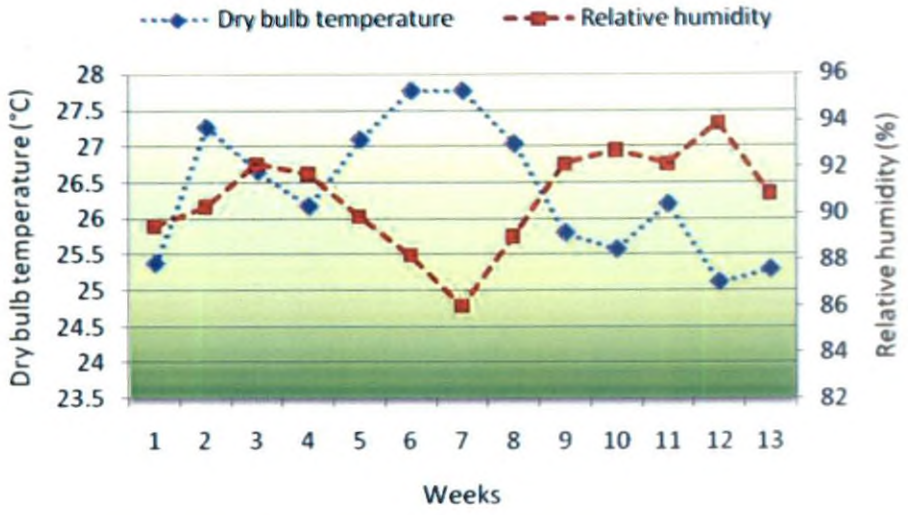


Fig. 8 - Weekly variation in dry bulb temperature (morning) and relative humidity (morning) during the experimental period

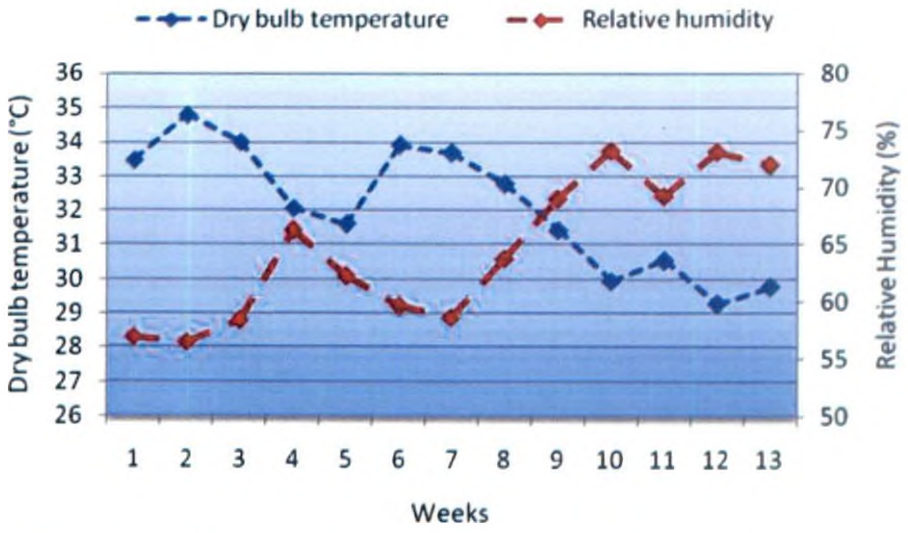


Fig. 9 - Weekly variation in dry bulb temperature (afternoon) and relative humidity (afternoon) during the experimental period

Table 4.4 Mean weekly Temperature Humidity Index (THI) values in the rabbitry

Weeks	THI morning	THI afternoon	THI Average
1	24.60±0.33	30.89±0.22	27.75±0.09
2	26.55±0.10	32.03±0.31	29.29±0.18
3	26.11±0.22	31.41±0.40	28.76±0.24
4	25.64±0.54	30.20±0.46	27.92±0.47
5	26.38±0.20	29.59±1.16	27.98±0.64
6	27.05±0.17	31.45±0.14	29.25±0.06
7	26.95±0.47	31.18±0.13	29.06±0.22
8	26.39±0.57	30.69±0.19	28.54±0.35
9	25.30±0.13	29.74±0.54	27.52±0.32
10	25.09±0.40	28.63±0.45	26.86±0.40
11	25.65±0.52	28.96±0.46	27.31±0.48
12	24.66±0.30	27.95±0.77	26.30±0.52
13	24.75±0.38	28.43±0.22	26.59±0.27

Table 4.5 Mean monthly Temperature Humidity Index in the rabbitry

Months	THI average
I	28.45±0.16
II	28.50±0.22
III	26.83±0.21

I- March, II-April, III-May

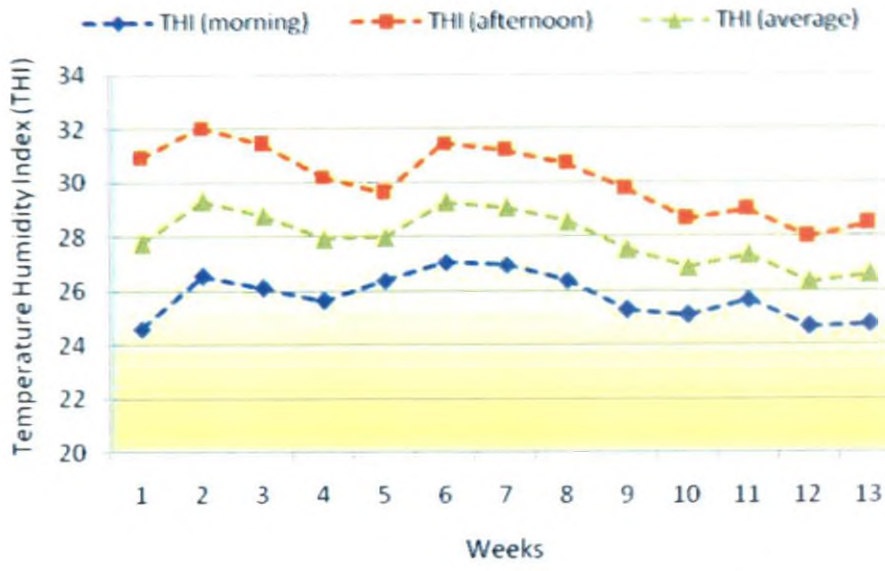


Fig. 10 - Mean weekly Temperature Humidity Index (THI) values during the experimental period

Fig. 11 - Mean monthly Temperature Humidity Index (THI) values during the experimental period

4.2 EFFECT OF CLIMATIC STRESS ON GROWTH PERFORMANCE OF NEW ZEALAND WHITE RABBITS

4.2.1 Mean weekly body weight

The mean weekly body weights of the rabbits during the experimental period are represented in Table 4.6 and Figure 12. The initial body weights of the animals in the four treatments viz. T1 (ascorbic acid supplemented group), T2 (probiotic supplemented roup), T3 (ascorbic acid and probiotic supplemented group) and T4 (control group) were 0.89±0.13kg, 0.92±0.13kg, 0.91±0.13kg and 0.91±0.11kg respectively and their final live body weights were 2.30±0.10kg, 2.41±0.11kg, 2.54±0.18kg and 1.92±0.18kg respectively. The initial body weight as well as body weights of the rabbits in the different treatment groups did not differ significantly ($P>0.05$) up to the tenth week. From the tenth week onwards the rabbits in T3 group had a significantly higher ($P\leq0.05$) body weight when compared to T4 rabbits. Also from the eleventh week onwards the animals in the T2 treatment had a significantly higher ($P\leq0.05$) body weight than T4 animals. No significant difference ($P>0.05$) was noted between T1 and T4 rabbits, but T1 animals had a numerically higher body weight compared to T4 animals. The rabbits in the T2 group (2.41 ± 0.11kg) and T3 group (2.54 ± 0.18kg) had significantly higher ($P\leq0.05$) and T1 group (2.30 ± 0.10kg) had numerically higher mean final body weights than animals in the T4 group (1.92 ± 0.18kg).

4.2.2 Mean daily body weight gain

The mean daily gain of New Zealand White rabbits is shown in Table 4.7 and Figure 13. The rabbits in the T1, T2 and T3 groups had higher body weight gains than T4 rabbits from the first week onwards. The treatment group T3 had significantly higher ($P\leq0.05$) body gain than T4 group from ninth week onwards. The overall mean

daily body weight gain were 15.43±0.57g (T1), 16.62±0.86g (T2), 17.95±0.75g (T3) and 11.14±0.34g (T4). The animals in the T1, T2 and T3 treatments had significantly higher ($P \leq 0.05$) overall mean body weight gain than T4 animals.

4.2.3 Mean daily feed intake

The average daily feed intake of the four treatment groups on dry matter basis is furnished in Table 4.8 and Figure 14. The overall mean daily feed intake of the four treatments were T1 (76.65±4.32g), T2 (79.50±4.3g), T3 (77.87±4.67g) and T4 (75.45±3.56g). No significant difference ($P>0.05$) was noted between the four treatment groups through out the experimental period. Numerically higher values for mean daily feed intake were obtained for T1, T2 and T3 animals compared to T4 animals.

4.2.4 Mean feed efficiency

Feed efficiency on dry matter basis under different treatments is presented in Table 4.9 and Figure 15. The animals in the T3 treatment had a significantly higher ($P \leq 0.05$) feed efficiency compared to T4 in the third week and ninth to thirteenth week. The overall mean feed efficiency of the treatments were T1 (6.02±0.33), T2 (5.42±0.33), T3 (4.72±0.15) and T4 (7.91±0.44). The animals in T1, T2 and T3 treatments had significantly higher ($P \leq 0.05$) overall mean feed efficiency compared to T4 animals.

Table 4.6 Mean weekly body weight of New Zealand White rabbits (kg)

week	T1	T2	T3	T4
Initial	0.89±0.13^{a}	0.92±0.13^{a}	0.91±0.13^{a}	0.91±0.11^{a}
1	1.02±0.13^{a}	1.07±0.15^{a}	1.06±0.14^{a}	1.00±0.10^{a}
2	1.11±0.13^{a}	1.16±0.15^{a}	1.17±0.15^{a}	1.08±0.15^{a}
3	1.21±0.13^{a}	1.25±0.15^{a}	1.27±0.16^{a}	1.15±0.10^{a}
4	1.33±0.14^{a}	1.37±0.17^{a}	1.40±0.17^{a}	1.23±0.10^{a}
5	1.44±0.13^{a}	1.52±0.16^{a}	1.50±0.17^{a}	1.30±0.17^{a}
6	1.53±0.12^{a}	1.63±0.16^{a}	1.61±0.17^{a}	1.39±0.17^{a}
7	1.64±0.11^{a}	1.70±0.14^{a}	1.71±0.16^{a}	1.46±0.16^{a}
8	1.77±0.10^{a}	1.86±0.15^{a}	1.85±0.15^{a}	1.55±0.15^{a}
9	1.87±0.10^{a}	1.97±0.15^{a}	1.99±0.16^{a}	1.62±0.16^{a}
10	1.96±0.10^{ab}	2.07±0.13^{ab}	2.10±0.16^{b}	1.70±0.16^{a}
11	2.06±0.10^{ab}	2.19±0.13^{b}	2.25±0.17^{b}	1.76±0.17^{a}
12	2.17±0.09^{ab}	2.30±0.11^{b}	2.39±0.18^{b}	1.86±0.18^{a}
13	2.30±0.10^{ab}	2.41±0.11^{b}	2.54±0.18^{b}	1.92±0.18^{a}

Mean values bearing different superscript in a row differ significantly ($P \leq 0.05$)

Table 4.7 Mean daily body weight gain of New Zealand White rabbits (g)

weeks	T1	T2	T3	T4
1	17.86±2.19^{a}	21.43±3.07^{a}	20.64±3.53^{a}	12.91±1.73^{a}
2	13.95±2.37^{a}	13.45±3.07^{a}	16.50±2.68^{a}	10.87±2.45^{a}
3	13.64±2.60^{a}	13.67±2.41^{a}	13.62±2.52^{a}	09.62±2.08^{a}
4	16.69±3.31^{a}	17.12±3.95^{a}	18.43±2.78^{a}	11.64±1.10^{a}
5	15.79±3.26^{ab}	21.36±2.94^{b}	15.38±3.74^{ab}	10.33±3.38^{a}
6	12.93±3.57^{a}	15.48±3.10^{a}	15.19±1.98^{a}	12.42±1.29^{a}
7	15.76±3.21^{a}	12.21±2.48^{a}	14.29±3.73^{a}	10.03±0.83^{a}
8	19.12±3.52^{ab}	21.64±2.27^{b}	19.57±1.41^{ab}	13.32±1.79^{a}
9	14.29±1.27^{ab}	16.33±1.77^{ab}	18.88±2.67^{a}	10.34±2.03^{b}
10	12.43±1.33^{ab}	14.95±3.18^{ab}	17.77±2.22^{a}	10.16±0.73^{b}
11	14.26±1.10^{ab}	15.93±2.15^{bc}	20.33±1.84^{bc}	09.81±1.93^{a}
12	16.02±2.57^{ab}	16.74±1.89^{ab}	21.67±3.44^{b}	11.90±2.52^{a}
13	17.81±3.88^{ab}	15.77±0.69^{ab}	21.07±1.76^{b}	11.48±3.13^{a}
Mean±SE	15.43±0.57^{a}	16.62±0.86^{ab}	17.95±0.75^{b}	11.14±0.34^{c}

Mean values bearing different superscript in a row differ significantly ($P \leq 0.05$)

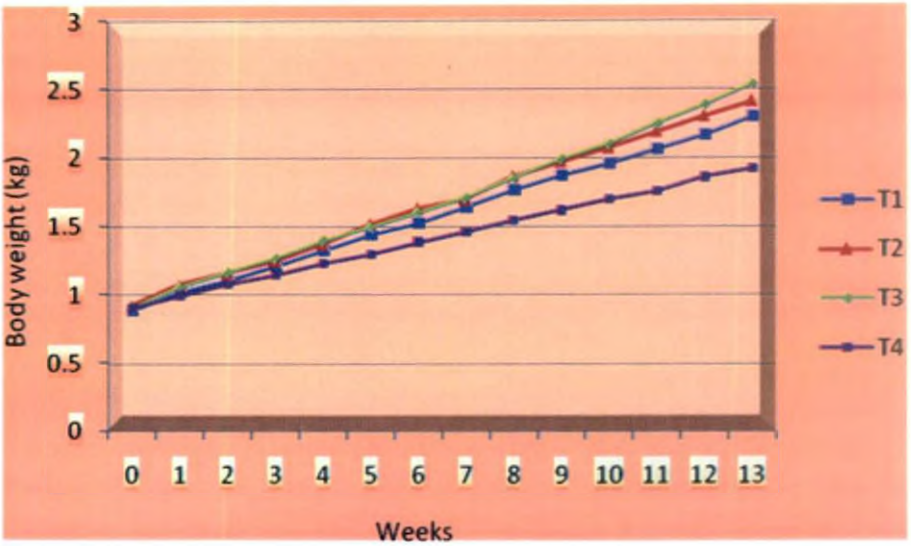


Fig. 12 - Mean weekly body weight of New Zealand White rabbits

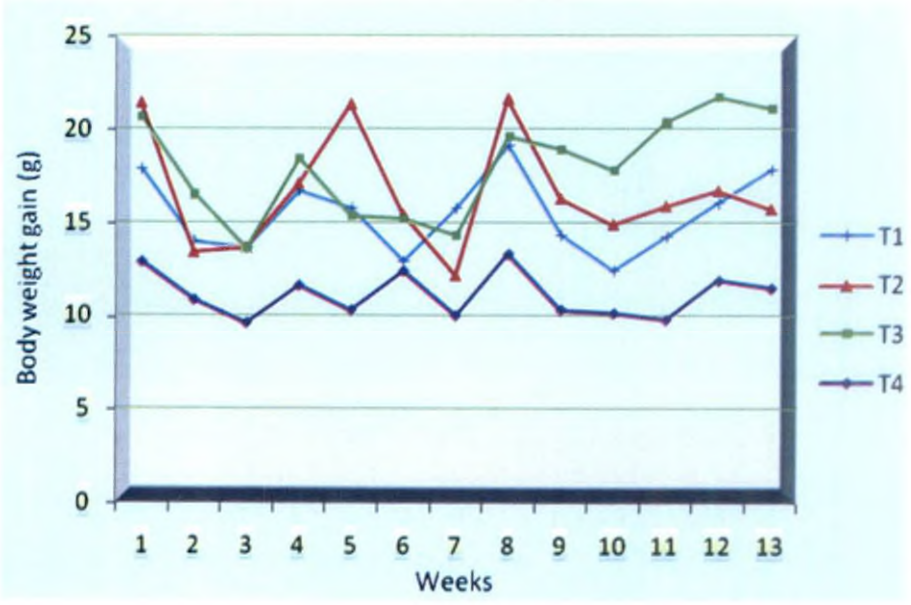


Fig. 13 - Mean daily body weight gain of New Zealand White rabbits

Table 4.8 Mean daily feed intake of New Zealand White rabbits (g)

Weeks	T1	T2	T3	T4
Initial	52.15±6.60	49.71±7.42	43.97±8.38	50.79±8.44
1	63.81±7.09	65.59±9.28	67.25±9.94	63.48±7.69
2	58.52±9.28	63.99±8.38	65.88±8.39	65.16±7.98
3	57.39±7.51	66.57±6.57	59.38±7.16	74.28±3.82
4	71.81±2.96	72.69±3.92	70.53±4.88	77.73±4.64
5	71.15±5.56	73.75±4.36	73.17±5.84	71.02±4.88
6	68.49±2.57	69.10±5.19	62.56±4.44	64.27±2.57
7	69.00±1.71	74.41±4.94	77.36±6.76	68.47±3.11
8	81.10±3.44	83.99±5.45	83.51±5.76	69.80±3.50
9	92.09±7.19	92.29±6.36	90.16±7.03	89.74±3.13
10	80.59±3.13	87.60±7.87	84.40±4.05	73.52±7.57
11	98.06±4.50	94.52±7.30	92.95±7.06	88.70±8.58
12	103.32±6.05	109.50±7.20	106.28±6.6	97.43±3.80
13	105.59±3.22	109.32±9.68	112.72±8.79	101.88±7.19
Mean±SE	76.65±4.32	79.50±4.30	77.87±4.67	75.45±3.56

Non significant ($P > 0.05$)

Table 4.9 Mean feed efficiency of New Zealand White rabbits

weeks	T1	T2	T3	T4
1	3.79±0.51^{ab}	3.20±0.42^{a}	3.76±0.70^{ab}	5.47±1.03^{b}
2	4.65±1.02^{a}	4.77±0.95^{a}	4.42±0.61^{a}	8.63±2.87^{a}
3	5.00±1.22^{a}	5.61±1.15^{ab}	4.78±0.56^{a}	9.92±2.39^{b}
4	6.49±2.65^{a}	5.88±1.75^{a}	4.23±0.58^{a}	7.27±1.35^{a}
5	6.35±2.11^{a}	3.89±0.76^{a}	4.83±4.19^{a}	8.07±2.68^{a}
6	5.49±4.99^{a}	4.48±2.29^{a}	4.44±0.57^{a}	5.57±0.82^{a}
7	6.02±1.80^{a}	6.09±3.42^{a}	5.61±3.63^{a}	7.19±0.94^{a}
8	4.71±1.84^{a}	4.16±0.56^{a}	4.45±0.56^{a}	5.59±0.58^{a}
9	6.76±0.87^{ab}	6.08±0.98^{ab}	5.32±0.82^{a}	9.93±3.79^{b}
10	6.98±0.96^{ab}	5.89±1.18^{ab}	4.28±0.24^{a}	8.81±1.14^{b}
11	7.06±0.51^{ab}	6.65±1.04^{ab}	4.90±0.49^{a}	9.18±1.19^{b}
12	7.41±1.40^{ab}	6.84±0.61^{ab}	4.56±0.47^{a}	9.91±1.72^{b}
13	7.60±2.84^{ab}	6.94±0.56^{ab}	5.09±0.72^{a}	9.25±5.13^{b}
Mean±SE	6.02±0.33^{a}	5.42±0.33^{ab}	4.72±0.15^{b}	7.91±0.44^{c}

Mean values bearing different superscript in a row differ significantly ($P \leq 0.05$)

Fig. 14 - Mean daily feed intake of New Zealand White rabbits

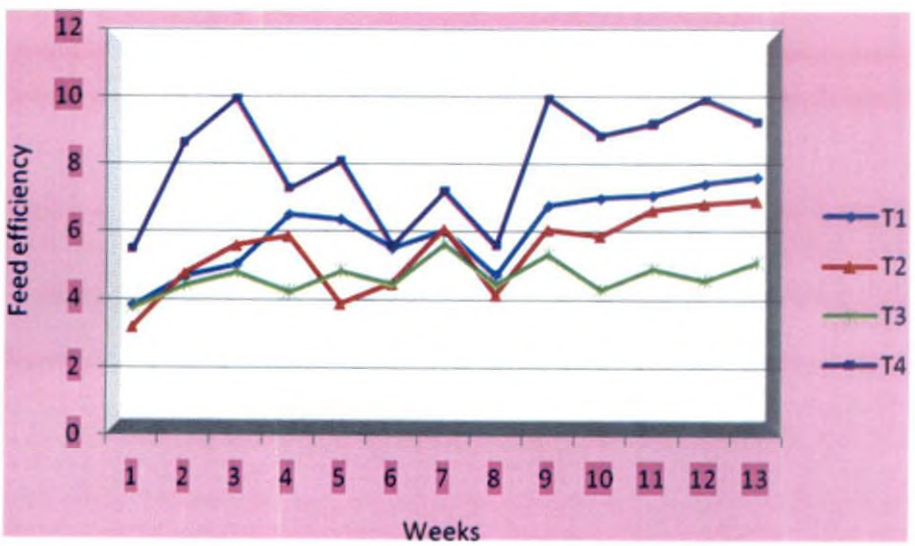


Fig. 15 - Mean feed efficiency of New Zealand White rabbits

4.3 EFFECT OF CLIMATIC STRESS ON PHYSIOLOGICAL RESPONSE OF NEW ZEALAND WHITE RABBITS

4.3.1 Mean weekly respiration rate

Mean weekly respiration rate (rates per minute) of the four treatments for the experimental period are furnished in Table 4.10 and Figure 16. Initially there was no significant difference ($P>0.05$) in respiration rate between the four treatments. However at the end of first week T2 rabbits had a significantly lower ($P\leq0.05$) respiration rate compared to the other treatments and from the first week to third week, fifth week and from the seventh week to tenth week T2 rabbits had a significantly lower ($P\leq0.05$) respiration rate compared to T4 rabbits. The rabbits in the T1 group had a significantly lower ($P\leq0.05$) respiration rate compared to T4 rabbits from the eighth week onwards. The animals in the T3 treatment had a numerically lower respiration rate compared to T4 animals from the second week onwards. The overall mean respiration rate (rates per min) of the four treatments viz T1, T2, T3 and T4 were 113.61±1.98, 113.23±1.16, 116.23±1.10 and 117.89±1.01 respectively. The rabbits in T1 and T2 groups had significantly lower ($P\leq0.05$) and T3 rabbits had numerically lower overall mean respiration rate compared to T4 rabbits while no significant difference ($P>0.05$) was there between T1, T2 and T3 rabbits.

4.3.2 Mean weekly rectal temperature

Mean weekly rectal temperature in °C of the four treatments for the experimental period are presented in Table 4.11 and Figure 17. The overall mean rectal temperature of the four treatments viz. T1, T2, T3 and T4 were 38.78±0.33°C, 38.92±0.47°C, 38.87±0.36°C and 39.00±0.42°C respectively. No significant

difference (P>0.05) was present among the four treatments. The rabbits in T1, T2 and T3 groups had numerically lower mean rectal temperature than T4 rabbits.

4.3.3 Mean monthly fecal cortisol levels

Mean monthly faecal cortisol values of the four treatments are presented in Table 4.12 and Figure 18. Initially there was no significant difference (P>0.05) between the four treatments. By the end of the first month the four groups began to differ significantly. The animals in the T1 and T3 treatments had a significantly lower faecal cortisol level in the first (P<.001) and the second month (P≤.05) compared to T4 animals, while T2 animals had a numerically lower cortisol level than T4 animals in the first two months. There was no significant difference (P>0.05) between the four treatments in the third month. The over all mean faecal cortisol values of the four treatments viz. T1, T2, T3, and T4 were 4.49±0.58µg/dl, 5.91±0.61µg/dl, 4.50±0.51µg/dl and 6.34±0.33µg/dl respectively. The rabbits in T1 and T3 groups had significantly lower (P≤0.05) and T2 rabbits had numerically lower overall mean faecal cortisol levels than T4 rabbits.

4.3.4 Mean monthly serum cortisol levels

The mean monthly serum cortisol values are furnished in Table 4.13 and Figure 19. The overall mean monthly serum cortisol values of the four treatments viz. T1, T2, T3 and T4 were 10.28±0.63µg per dl, 9.95±0.72µg per dl, 10.37±0.10µg per dl and10.47±0.64µg per dl. No significant difference (P>0.05) in serum cortisol was observed between the four treatments during the experimental period. The animals in the T1, T2 and T3 treatment had numerically lower overall mean serum cortisol values than T4 animals.

Table 4.10 Mean weekly respiration rate of New Zealand White rabbits (cycles per min)

week	T1	T2	T3	T4
Initial	122.67±1.31^{a}	121.00±1.32^{a}	123.33±0.88^{a}	123.17±0.75^{a}
1	120.33±0.56^{b}	116.33±1.61^{a}	119.83±1.0^{b}	120.83±0.48^{b}
2	124.00±1.00^{ab}	121.50±1.15^{a}	123.83±0.87^{ab}	125.67±0.95^{b}
3	119.50±0.50^{ab}	117.17±1.56^{a}	120.17±0.54^{ab}	122.17±0.95^{b}
4	120.33±1.56^{a}	116.83±1.50^{a}	120.00±1.46^{a}	121.00±1.88^{a}
5	119.50±0.22^{b}	114.83±1.60^{a}	117.67±0.61^{ab}	119.33±1.33^{b}
6	115.33±0.88^{a}	112.50±1.26^{a}	113.50±1.02^{a}	116.17±1.68^{a}
7	114.83±1.56^{b}	109.00±2.01^{a}	113.83±1.33^{ab}	114.50±1.69^{b}
8	109.67±2.35^{a}	110.33±1.09^{a}	113.50±1.02^{ab}	115.83±1.35^{b}
9	109.00±1.97^{a}	109.33±1.12^{a}	112.67±1.26^{ab}	115.67±1.61^{b}
10	108.17±1.87^{a}	110.17±1.33^{a}	112.50±1.18^{ab}	115.50±1.88^{b}
11	106.17±1.68^{a}	109.67±2.55^{ab}	113.00±1.21^{b}	115.17±1.74^{b}
12	99.33±1.74^{a}	108.67±2.86^{b}	112.33±1.73^{b}	113.33±3.75^{b}
13	101.67±0 .61^{a}	107.83±2.63^{ab}	111.00±1.32^{b}	112.17±3.18^{b}
Mean±SE	113.61±1.98^{a}	113.23±1.16^{a}	116.23±1.10^{ab}	117.89±1.01^{b}

Mean values bearing different superscript in a row differ significantly ($P \leq 0.05$)

Table 4.11 Mean weekly rectal temperature of New Zealand White rabbits (°C)

week	T1	T2	T3	T4
Initial	38.68±0.14	38.69±0.08	38.65±0.11	38.62±0.08
1	39.28±0.10	39.32±0.20	39.42±0.09	39.62±0.11
2	39.38±0.11	39.58±0.18	39.62±0.09	39.65±0.14
3	39.13±0.08	39.63±0.08	39.20±0.14	39.68±0.16
4	39.10±0.11	39.57±0.09	39.15±0.22	39.22±0.15
5	38.67±0.15	38.57±0.18	38.27±0.09	38.83±0.09
6	38.72±0.12	38.52±0.13	38.37±0.11	38.67±0.14
7	38.43±0.07	38.90±0.11	38.67±0.25	38.53±0.12
8	38.53±0.03	38.75±0.13	38.70±0.14	38.93±0.05
9	38.67±0.04	38.88±0.11	38.93±0.22	39.12±0.04
10	38.85±0.14	38.97±0.11	38.73±0.12	39.20±0.18
11	38.12±0.11	37.88±0.15	38.75±0.19	38.25±0.13
12	38.72±0.24	38.67±0.22	38.95±0.13	38.75±0.15
13	38.65±0.03	38.93±0.14	38.72±0.15	38.98±0.14
Mean±SE	38.78±0.33	38.92±0.47	38.87±0.36	39.00±0.42

Non significant (P>0.05)

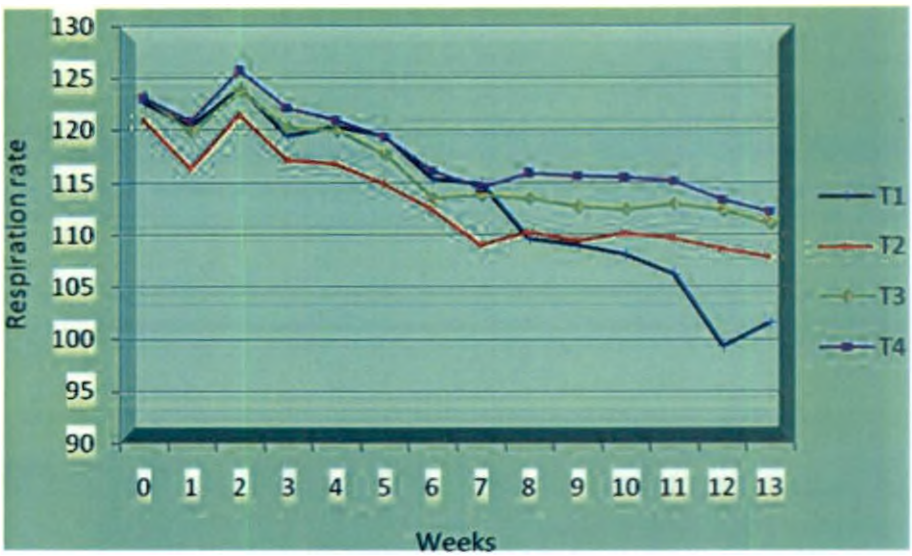


Fig. 16 - Mean weekly respiration rate of New Zealand White rabbits (cycles per min)

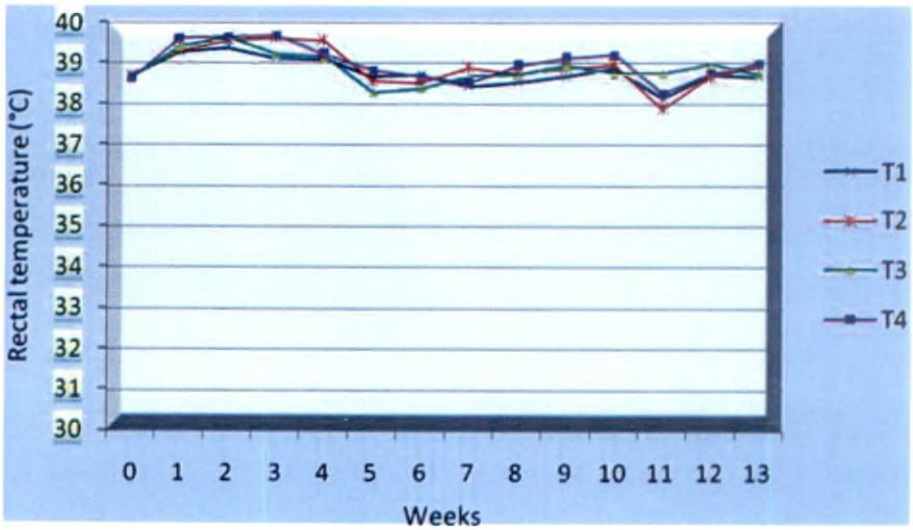


Fig. 17 - Mean weekly rectal temperature of New Zealand White rabbits

Table 4.12 Mean monthly fecal cortisol values of New Zealand White rabbits (µg/dl)

Months	T1	T2	T3	T4
Initial	5.94±1.27^{a}	6.18±1.45^{a}	5.34±0.94^{a}	5.95±1.91^{a}
1	2.48±1.15^{a}	5.88 ±1.33^{b}	3.56 ±0.77^{a}	6.12±1.85^{b}
2	5.21±0.76^{a}	5.97±2.13^{ab}	5.49±1.80^{a}	7.58±0.15^{b}
3	4.31±2.99^{a}	5.61±0.28^{a}	3.62±0.87^{a}	5.70±1.41^{a}
Mean±SE	4.49±0.58^{a}	5.91±0.61^{b}	4.50±0.51^{a}	6.34±0.33^{b}

Mean values bearing different superscript in a row differ significantly ($P<.001$ or $P\leq.05$)

Table 4.13 Mean monthly serum cortisol values of New Zealand White rabbits (µg/dl)

Months	T1	T2	T3	T4
Initial	12.15±0.69	12.19±0.50	10.74±1.05	10.57±0.49
1	9.45±1.06	9.72±0.62	10.33±0.58	10.86±0.50
2	10.92±1.61	9.68±0.60	10.54±0.37	11.15±0.49
3	10.28±0.63	9.95±0.72	10.37±0.10	10.47±0.64
Mean±SE	10.70±0.28	10.39±0.30	10.50±0.05	10.76±0.08

Non significant ($P>0.05$).

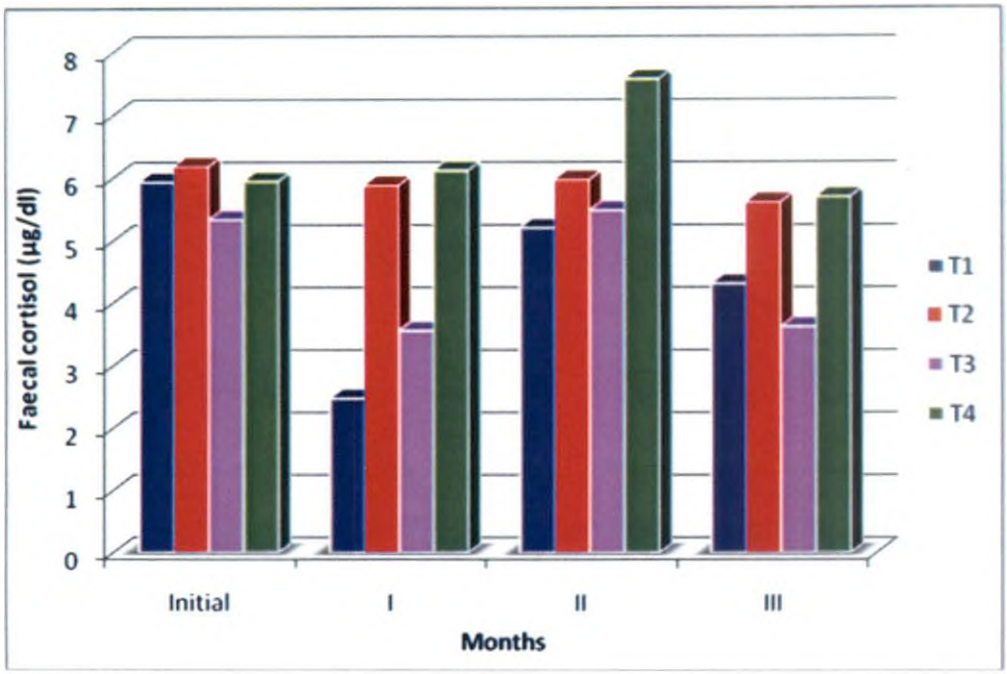


Fig. 18 - Mean monthly faecal cortisol values of New Zealand White rabbits

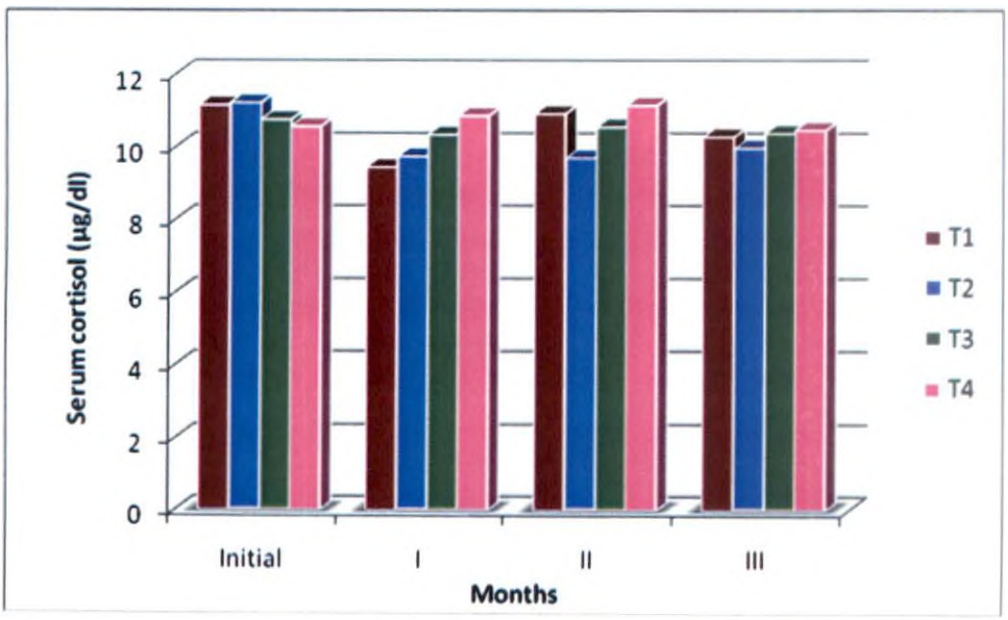


Fig. 19 - Mean monthly serum cortisol values of New Zealand White rabbits

4.4 DISEASE INCIDENCE AND MORTALITY

Incidence of any diseases or mortality was not reported during the experimental period.

4.5 COST EFFECTIVENESS OF SUPPLEMENTING PROBIOTIC AND ASCORBIC ACID

The cost effectiveness of supplementing probiotic and ascorbic acid were determined and the results are presented in Table 4.15 and Figure 20. The animals in the T3 group recorded the lowest cost of production with Rs. 76.28 per kg live weight, followed by T2 (79.67), T1 (96.08) and T4 (114.70).

Table 4.14. Cost of production per kg live weight of New Zealand White rabbits (on feed basis)

Treatments	T1	T2	T3	T4
Number of rabbits	6	6	6	6
Total initial body weight (kg)	5.34	5.52	5.46	5.46
Total final body weight (kg)	13.8	14.46	15.24	11.52
Total body weight gain (kg)	8.46	8.94	9.78	6.06
Total feed intake (kg)	42.88	44.66	43.94	42.23
Total feed cost (Rs.)	684.21	656.5	709.9	612.34
Cost of feed per kg (Rs.)	15.96	14.7	16.16	14.5
Feed efficiency	6.02	5.42	4.72	7.91
Cost of production on feed basis (Rs.)	96.08	79.67	76.28	114.70

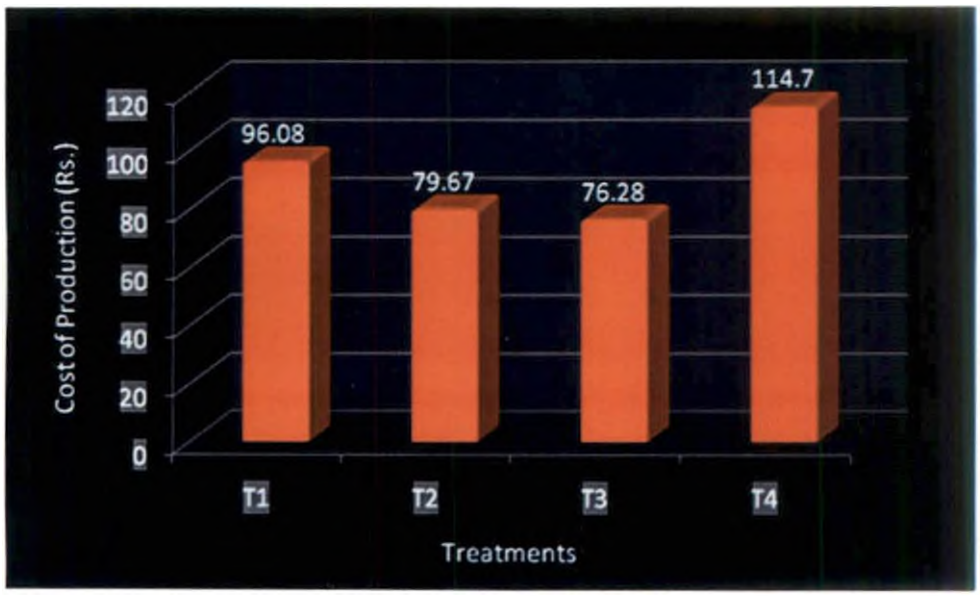


Fig. 20 - Cost of production per kg live weight of New Zealand White rabbits (on feed basis)

Discussion

5. DISCUSSION

5.1 ASSESMENT OF STRESS

5.1.1 Macro and micro climatic changes

The macro and micro climatic variables (Table 4.1) like mean monthly maximum temperature, minimum temperature, and relative humidity (morning and afternoon) showed no significant difference ($P>0.05$). The mean monthly maximum temperature in the rabbitry was highest in March (34.12±0.03°C) and lowest in May (30.01±0.04°C). The mean monthly minimum temperature in the rabbitry was highest in April (25.6±0.02°C) and lowest in May (24.1±0.02°C). The relative humidity was highest in May both in the morning (92.68±0.07%) and afternoon (71.19±0.33%).

The data regarding the mean weekly dry bulb temperature (afternoon) (Table 4.2) showed that it was highest (34.79±0.45°C) in the second week of the experiment and lowest (29.26±1.02°C) in the 12^{th} week of the experiment. The dry bulb temperature (morning) was highest (27.77±0.17°C) in the sixth and seventh week of the experiment and lowest (25.11±0.30°C) in the 12^{th} week. The results on the mean weekly relative humidity values (Table 4.3) showed that it was highest in the 12^{th} week of the experiment (93.86±0.91% in the morning and 73.19±4.52% in the afternoon).

The mean weekly temperature humidity index (THI) values (Table 4.4) were higher in the afternoon compared to the morning. According to Marai *et al.* (2001), in rabbit, temperature humidity values less than 27.8 indicated absence of heat stress, 27.8 to 28.9 indicated moderate heat stress, 28.9 to 30 indicated severe heat stress and values greater than 30.0 indicated very severe heat stress. Accordingly, it was evident that in the present study severe stress prevailed in the second (29.29±0.18), sixth (29.25±0.06) and seventh week (29.06±0.22) of the experimental period and

moderate stress prevailed in the third (28.76±0.24), fourth (27.92±0.47), fifth (27.98±0.64) and eighth (28.54±0.35) week of the experimental period.

The mean monthly temperature humidity index values (Table 4.5) suggested that animals were under moderate heat stress in the first (28.45±0.16) and the second month (28.50±0.22), while no stress prevailed in the third month (26.83±0.21). In the 12th week of the experiment the dry bulb temperature was lowest (both in the morning and afternoon) and relative humidity was highest (both in the morning and afternoon) and the temperature humidity index (THI) value was lowest which indicates that temperature had got a profound influence regarding stress in rabbits.

5.2 EFFECT OF CLIMATIC STRESS ON GROWTH PERFORMANCE OF NEW ZEALAND WHITE RABBITS

5.2.1 Mean weekly body weight

Mean weekly body weights (Table 4.7) of different treatments showed that there was a significant ($P \leq 0.05$) difference among different treatments towards the end of the experimental period. Body weight of rabbits in T3 group was significantly ($P \leq 0.05$) higher than that of T4 rabbits from tenth week onwards. Mean final body weight of T1, T2, T3 and T4 animals were 2.30±0.10kg, 2.41±0.11kg, 2.54±0.18kg and 1.92±0.18kg respectively. The rabbits in the T3 treatment had the highest final mean weight among the four treatments. This was in agreement with the research work done by Abd-Elhalim, (2008). Also, the animals in T2 group had significantly higher ($P \leq 0.05$) body weight than T4 animals from the eleventh week onwards. This observation was similar to the results of Amber *et al.* (2004), while this contradicted the results of Kapila *et al.* (2006) who stated that supplementation of the probiotic, *Lactobacillus casei* did not have any effect on the body weight of rabbits. Marai *et al.* (2001) reported that digestibility coefficients declined due to heat stress by 7.9 per

cent in dry matter, 8.1 per cent in crude protein and 1.0 per cent in crude fibre. Supplementation of *Lactobacilli* improved digestibility of dry matter, crude protein, ether extract and crude fiber in rabbits and the increase in nutrient digestibility might be due to reduction in the surface tension of cell membranes by bacteria favoring better absorption of nutrients across the cell membranes, also the improved digestibility of crude fiber might be due to increase in the gut cellulolytic bacterial population as a result to enhancing lactate utilization and moderating pH of the media (Amber *et al.* 2004). Hence the higher body weight in T2 rabbits compared to T4 rabbits might be attributed to the improved digestibility of nutrients resulting in better feed efficiency. Ascorbic acid also had a role in slightly improving the digestibility of nutrients. (Yacout *et al.* 2002). There was no significant difference in body weight between T1 and T4 animals, but T1 animals had a numerically higher body weight than T4 animals suggesting that supplementation of ascorbic acid alone did not have a significant effect on the body weight of rabbits. This observation was supported by the view of Konca *et al.* (2009) in chicken. Hence from the current study it could be concluded that probiotic alone or in combination with ascorbic acid had a positive effect in improving the body weight of rabbits under heat stress.

5.2.2 Mean daily body weight gain

The daily body weight gain values (Table 4.7) of New Zealand White rabbits revealed that T1, T2 and T3 animals had higher body weight gain than T4 animals from the first week onwards. Significant difference ($P \leq 0.05$) was noted between T3 and T4 rabbits from the ninth week onwards. The overall mean daily body weight gain were 15.43±0.57g (T1), 16.62±0.86g (T2), 17.95±0.75g (T3) and 11.14±0.34g (T4) respectively. The rabbits in the T1, T2 and T3 treatments showed significantly higher overall mean daily body weight gain compared to T4 rabbits. This coincided with the observations of Selim *et al.* (2008) who stated that dietary supplementation

of 200 ppm of ascorbic acid significantly increased live weight gain in New Zealand White rabbits and Amber *et al.* (2004) who reported that supplementation of *Lactobacilli* increased the average daily gain in New Zealand White rabbits. However the present study was contrary to the findings of Kamra *et al.* (1996) who proposed that probiotic, *Lactobacillus casei* did not have any significant effect on the daily body weight gain of rabbits and Konca *et al.* (2009) in chicken, according to whom ascorbic acid alone did not significantly affected daily body weight gain. The increased body weight gain in T3 rabbits in the current study might be due to the high feed efficiency as a result of the improved digestibility of nutrients due to the combined effect of probiotic and ascorbic acid.

5.2.3 Mean daily feed intake

The mean daily feed intake (Table 4.8) of the four treatments did not differ significantly ($P>0.05$) through out the experimental period. This was supported by the results of Skiivanova *et al.* (1998) who stated that dietary supplementation of ascorbic acid did not affect feed intake in rabbits reared at high temperature. According to the study done by Kamra *et al.* (1996), supplementation of *Lactobacilllus casei* did not have any effect on the feed intake of rabbits under heat stress. Several studies had revealed that high summer temperature significantly reduced feed intake in rabbits (Cheiricato *et al.* (1993), Fernandez *et al.* (1994), Marai *et al.* (1999) and Bovera *et al.* (2008)). In the present study, no significant difference ($P>0.05$) in feed intake was noted between the control group and feed supplemented groups, but significant difference was noted in other growth performance traits. The reason might be that the reduction in feed intake due to high summer temperature in the hotter parts of the day would have been compensated by increased feed intake during the cooler hours of the day ie, the night hours. Hence it can be inferred that supplementation of ascorbic acid or probiotic did not have any

effect on the feed intake of New Zealand White rabbits reared under high temperature.

5.2.3 Feed Efficiency

The results on the feed efficiency on dry matter basis (Table 4.9) revealed that there was significant ($P \leq 0.05$) difference in feed efficiency between the different treatments. The overall mean feed efficiency of T1 (6.02±0.33), T2 (5.42±0.33) and T3 (4.72±0.15) rabbits were higher compared to T4 (7.91±0.44) rabbits while the rabbits in T3 group had significantly higher ($P \leq 0.05$) feed efficiency compared to T4 rabbits from the ninth week onwards. These results were similar to that of Abd-Elhalim (2008) who reported that ascorbic acid and probiotic (Roemin W2®) addition in the diet significantly improved feed conversion efficiency in growing New Zealand White rabbits. As per Das *et al.* (1991) feed efficiency was negatively correlated with both air temperature and relative humidity. Cheiricato *et al* (1993) reported that higher temperature resulted in poor feed efficiency in rabbits and Marai *et al.* (2001) observed that high summer temperature resulted in poor digestibility of nutrients in rabbits. Thus the poor feed efficiency in the animals of T4 group in the present study might be due to high temperature stress as a result of the poor digestibility of nutrients. The improved feed efficiency in T1, T2 and T3 rabbits might be due to better feed utilization as a result of improved digestibility of feed ingredients.

5.3 EFFECT OF CLIMATIC STRESS ON PHYSIOLOGICAL RESPONSE OF NEW ZEALAND WHITE RABBITS

5.3.1 Mean respiration rate

The mean weekly respiration rate (Table 4.11) of the different treatments revealed that T1 and T2 rabbits had significantly ($P \leq 0.05$) lower overall mean

respiration rate than T4 rabbits. This is in conformity with the results of Abdel-Samee, (1955). Hence supplementation of probiotic, *L. casei* and ascorbic acid can reduce the heat load in rabbits as observed by reduction in the respiration rate. The animals in T3 group had a numerically lower respiration rate compared to T4 animals from the second week onwards and no significant difference was noted between T1, T2 and T3 animals. The overall mean respiration rate of the four treatments viz. T1, T2, T3 and T4 were 113.61±1.98 cycles per min, 113.23 ±1.16 cycles per min, 116.23±1.10 cycles per min and 117.89±1.01 cycles per min. The mean values recorded for respiration rate in all the four treatments were higher than the values reported by Marai *et al.* (1996) [108.5±1.4] while the values were lower than that recorded by Marai *et al.* (1994b) [133.0]. This difference in values might be due to the difference in climatic variables prevailing in the concerned locations. Several researchers found that respiration rate in rabbits increased under heat stress conditions [Marai *et al.* (1991) and Ogunjimi *et al.* (2008)]. Since most of the sweat glands in rabbits are not functional and perspiration is never great because of the fur, the only controlled means of latent heat evacuation is by altering the breathing rate (Marai *et al.* 1991). In the present study also, the control group (T4) had a numerically higher respiration rate compared to the other three groups. The highest value for respiration rate in T4 rabbits was obtained in the second (125.67 ± 0.95 cycles per min) week of the experimental period when the dry bulb temperature (second week (34.79±0.45°C) reached peak levels. This supported the view of Richards, (1976) who stated that respiratory frequency increases with increases in ambient temperature above panting threshold. The significance of the increase in respiration is that it enables the animal to dissipate heat by vaporizing the moisture through the respiratory air, which accounts for about 30 per cent of the total heat dissipation (Maclean, 1963). This system work between 0 and 30°C and at this range of temperature latent heat evacuation is only controlled by altering the breathing rate

(Marai *et al.* 1994a). However, dissipation of heat through respiratory passage was decreased by increase in ambient humidity (Lebas *et al.* 1986). Thus from this study it could be inferred that the respiration rate in New Zealand White rabbits was influenced by environmental temperature and supplementation of *L. casei* and ascorbic acid could reduce the heat load in rabbits under stress.

5.3.2 Mean rectal temperature

The mean weekly rectal temperature (Table 4.11) showed no significant difference ($P>0.05$) among the four treatments. The mean rectal temperature of the four treatments viz. T1, T2, T3 and T4 were 38.78±0.33°C, 38.92±0.47°C, 38.87±0.36°C and 39.00±0.42°C. Finzi *et al.* (1994) reported that the average body temperature in rabbits goes up from morning till night, while environmental air temperature goes up from morning till noon then decreases at night, indicating that body temperature was not affected instantly by changes in air temperature during the day. Shafie *et al.* (1970) observed that the diurnal variation in body temperature varied within a very short range (0.2–0.3 °C). The study done by Ogunjimi *et al.* (2008) revealed that a low correlation existed between rectal temperature and thermal comfort level, while a strong correlation existed between respiration rate and thermal comfort level in rabbits in rabbits. In the present study no between group significant ($P>0.05$) difference was noted for rectal temperature but other physiological parameters like respiration rate and faecal cortisol varied significantly suggesting that rectal temperature was not an indicator of heat stress in rabbits. Hence it could be concluded that supplementation of probiotic, *L. casei* and ascorbic acid did not exert a significant effect on the rectal temperature of rabbits reared under high temperature.

5.3.2 Mean faecal cortisol values

The mean monthly faecal cortisol values (Table 4.12) in the four treatments had significant difference in the first ($P<0.001$) and the second month ($P\leq0.05$). Faecal cortisol level of animals in T1 and T3 groups were significantly lower than T4 animals in the first ($P<0.001$) and second month ($P<0.05$) while T2 animals had a numerically lower value compared to T4 animals in first two months. This finding was similar to the observations made in chicken by Karthiayini *et al.* (2007) for serum cortisol, according to whom Vitamin C produced a significant reduction but probiotic supplementation could only produce a numerical reduction in the cortisol level during heat stress. No significant difference in faecal cortisol level was noticed between the different treatments in the third month but T1, T2 and T3 rabbits had numerically lower values than T4 rabbits in the third month. As per the study of Huber *et al.* (2003) out of several potential predictor variables investigated minimum ambient temperature was an important factor exerting a significant effect on fecal glucocorticoid excretion. In the current study also, the highest values for faecal cortisol in all the treatments were obtained in the second month when the minimum ambient temperature was the highest (25.17±0.05°C). Considering the control group alone, the lowest value for faecal cortisol was obtained in the third month (5.70±1.41 µg per dl) compared to the first (6.12± 1.85 µg per dl) and second month (7.58±0.15 µg per dl). Thus it could be inferred that level of stress was more in the first two months compared to the third month. This could be correlated to the higher temperature humidity index values in the first (28.45±0.16) and second (28.50±0.22) month and lower temperature humidity index value in the third month (26.83±0.21). Hence it could be concluded that level of faecal cortisol was influenced by temperature and humidity. Furthermore, supplementation of probiotic, *L. casei* in combination with ascorbic acid had a positive influence in alleviating summer stress in New Zealand White rabbits.

5.3.3 Mean monthly serum cortisol values

The mean monthly serum cortisol values (Table 4.13) suggested that the four treatments did not differ significantly during the entire experimental period. The animals in the T1, T2 and T3 group had numerically lower mean monthly serum cortisol levels than T4 animals. This finding was contradictory to the observations made in chicken by Karthiayini *et al.* (2007). Some studies showed that blood glucocorticoids either increase (Satterlee *et al.* 1977), decrease (Collier *et al.* 1982) or are not affected (El-Nouty *et al.*, 1980) by heat stress. The literature dealing with the effect of hot climate on blood cortisol level was rather conflicting (Marai *et al.* 2002). In the present study significant difference was observed for faecal cortisol but not for serum cortisol. Blood collection for estimation of cortisol was done in the morning and the temperature humidity index values in the morning were lower than 27.8 indicating absence of stress in rabbits in the morning while the THI values were high in the afternoon. The lack of stress in the morning hours might be the logical reason for not showing any significant difference in serum cortisol among the four treatments.

5.4 DISEASE INCIDENCE AND MORTALITY

Disease incidence or mortality was not observed during the experimental period. This is contrary to the findings of Devi *et al.* (1990) who stated that heavy rabbit mortality due to pasteurellosis was seen during the period from March to June. Many authors reported incidence of diseases in rabbits during hot summer [Patton (1984) and Nandakumar (1995)]. Temperature humidity index values above 30 indicated very severe stress in rabbits (Marai *et al.* 2001). As per the results obtained for faecal cortisol and temperature humidity index values, the animals were subjected to stress in the first and second month while no stress prevailed in the third month in this study. The mean temperature humidity index values never exceeded very severe

levels (> 30). The difference in results in the present study to that of Devi *et al.* (1990) and Nandakumar (1995) might be due to the lower level of stress in this study.

5.5 COST EFFECTIVENESS OF SUPPLEMENTING PROBIOTIC AND ASCORBIC ACID

The cost of production is the basic measure of economic efficiency in rabbit husbandry (Biya (2006)). Since feed is the major factor contributing to the cost of production, the economics had been calculated based on feed cost. Cost of production per kilo gram live weight on feed basis for the different treatment groups is presented in Table 4.14. The cost of production (Rupees) was lowest for T3 (76.28), followed by T2 (79.67), T1 (96.08) and T4 (114.70). These observations were supported by the findings of Abd-Elhalim (2008) who stated that supplementation of probiotic improved the economic efficiency of New Zealand White rabbits. Many authors reported that the economic gain from rabbits was low during higher environmental temperature (Bovera *et al.* (2008) Marai *et al.* (1999) and Yamani and Khalil (1994)). Hence it could be concluded supplementation of probiotic, *Lactobacillus casei* and ascorbic acid in feed had a positive effect in improving the economic efficiency in rabbit production.

From the current study it could be concluded that supplementation of probiotic and ascorbic acid can be effectively and economically used to alleviate summer stress in growing broiler rabbits.

Summary

6. SUMMARY

The research work was conducted to study the efficacy of probiotic (*Lactobacillus casei*) and ascorbic acid in alleviating summer stress in growing broiler rabbits. The study was done in the summer season from March to May. The growth performance of rabbits in summer, their physiological response to stress and the cost effectiveness of supplementing probiotic, *Lactobacillus casei* and ascorbic acid were studied.

Twenty four weaned New Zealand White rabbits were randomly selected from Rabbit unit at Krishi Vigyan Kendra, Kerala Agricultural University, Vellanikkara as uniformly as possible with respect to age and body weight and were utilized for the study. The animals were allotted randomly to the experimental treatments and each of the four treatments was replicated six times. The treatments were as follows: Treatment I (T1) - Ascorbic acid (Merck) at the rate of 200 mg per kg feed was given along with the basal diet, Treatment - 2 (T2) - Probiotic, *Lactobacillus casei* (Unique Biotech) containing 10^6 colony forming units per gram of feed was given along with the basal diet, Treatment - 3 (T3) - Probiotic and Ascorbic acid at the same rate as in T1 and T3 were given along with the basal diet and Treatment - 4 (T4) - Rabbits fed with basal diet alone.

The various parameters studied included maximum and minimum temperature and relative humidity (morning and afternoon) in macro climate, maximum and minimum temperature, dry bulb temperature (morning and afternoon) and relative humidity (morning and afternoon) in micro climate, production parameters like weekly body weight, daily feed intake, average daily body weight gain and feed efficiency, physiological parameters like weekly respiration rate, weekly rectal temperature and monthly cortisol levels (faecal and serum) (twice a month for three months). The disease incidence and mortality in the rabbitry

during the period was recorded. Cost effectiveness of supplementing probiotic and ascorbic acid was determined. The results were analyzed using appropriate statistical tools.

By one way Analysis of variance it was found that supplementation of probiotic, *Lactobacillus casei* and ascorbic acid had significant effect in alleviating summer stress in rabbits. The treatments groups differed significantly in mean weekly body weight, mean daily body weight gain, feed efficiency, respiration rate and faecal cortisol level. There was no significant difference between the treatments in mean daily feed intake, rectal temperature and serum cortisol level.

The changes in environmental variables all throughout the study period revealed that the animals were exposed to stress during March and April. No significant difference ($P>0.05$) existed between macro and micro climatic variables during the experimental period. The mean monthly maximum temperature in the rabbitry was highest in March (34.12±0.04°C) and lowest in May (30.01±0.04°C). The mean monthly minimum temperature in the rabbitry was highest in April (25.6±0.02°C) and lowest in May (24.1±0.02°C). The relative humidity was highest in May both in the morning (92.68±0.07%) and afternoon (71.19±0.33%). The mean weekly temperature humidity index (THI) values were higher in the afternoon compared to the morning. The mean monthly temperature humidity index values suggested that animals were under moderate heat stress in the first (28.45±0.16) and the second month (28.50±0.22), while no stress prevailed in the third month (26.83±0.21). The temperature humidity index (THI) value was lowest in the 12^{th} week of the experiment when the dry bulb temperature was lowest and relative humidity was highest.

Mean weekly body weights of different treatments showed that there was a significant ($P\leq0.05$) difference among them towards the end of the experimental period. The rabbits in the T3 group was significantly ($P\leq0.05$) higher than T4 rabbits

from the tenth week onwards. The T3 animals had the highest final mean weight among the four treatments. The T2 animals had significantly ($P \leq 0.05$) higher body weight than T4 animals from the eleventh week onwards. There was no significant difference between T1 and T4, but T1 had a numerically higher body weight than T4.

The mean daily body weight gain of New Zealand White rabbits revealed that T1, T2 and T3 animals had higher body weight gains than T4 from the first week onwards. Significant difference ($P \leq 0.05$) was there between T3 and T4 animals from the ninth week onwards. The T1, T2 and T3 rabbits had significantly ($P \leq 0.05$) higher overall mean body weight gain than T4.

Dietary supplementation of probiotic, *Lactobacillus casei* and ascorbic acid did not make a significant difference ($P > 0.05$) in the feed intake of rabbits reared in summer. The T1, T2 and T3 rabbits recorded numerically higher overall mean values compared to T4 rabbits.

The feed efficiency on dry matter basis among the four treatments differed significantly ($P \leq 0.05$). The T3 animals had significantly ($P \leq 0.05$) higher feed efficiency compared to T4 animals from the ninth week onwards. The T1 (6.02 ± 0.33), T2 (5.42 ± 0.33) and T3 (4.72 ± 0.15) rabbits had significantly higher overall mean feed efficiency compared to T4 (7.91 ± 0.44) rabbits.

The mean weekly respiration rate of the different treatments revealed that T1 and T2 animals had significantly ($P \leq 0.05$) lower and T3 animals had numerically lower overall mean respiration rate compared to T4 animals. The T2 rabbits had a significantly ($P \leq 0.05$) lower respiration rate compared to T4 rabbits from the first week to third week, fifth week and from the seventh week to tenth week. The T1 animals had a significantly ($P \leq 0.05$) lower respiration rate compared to T4 from the eighth week onwards. The highest values for respiration rate in T4 group was

obtained in the second (125.67 ± 0.95 cycles per min) of the experimental period when the dry bulb temperature (second week (34.79±0.45°C) reached peak levels.

The supplementation of probiotic, *Lactobacillus casei* and ascorbic acid did not exert a significant effect on the mean weekly rectal temperature of New Zealand White rabbits reared in summer season. The mean rectal temperature values of T1, T2 and T3 were comparatively lower than T4.

The mean monthly faecal cortisol values in the four treatments had significant difference in the first ($P<0.001$) and the second month ($P\leq0.05$). The animals in T1 and T3 group were significantly lower than T4 in the first ($P<0.001$) and second month ($P\leq0.05$) while T2 animals had a numerically lower value compared to T4 in first two months. No significant difference ($P\leq0.05$) in faecal cortisol existed between the different treatments in the third month but T1, T2 and T3 rabbits had numerically lower faecal cortisol values than T4 rabbits in the third month. The highest values for faecal cortisol in all the treatments were obtained in the second month when the minimum ambient temperature was the highest (25.17±0.05°C). Considering the control group alone, the lowest value for faecal cortisol was obtained in the third month (5.70±1.41µg per dl) compared to the first (6.12± 1.8541µg per dl) and second month (7.58±0.1541µg per dl) when the temperature humidity index was lowest. The mean monthly serum cortisol values suggests that the treatments did not differ significantly ($P>0.05$) during the entire study period.

Disease incidence or mortality was not observed during the experimental period. The cost effectiveness of supplementing probiotic and ascorbic acid were determined. Supplementation of probiotic and ascorbic acid was found to be efficient in reducing the production economics. Hence it was concluded that supplementation of probiotic, *Lactobacillus casei* at the rate of 10^6 cfu per g of feed and ascorbic acid at the rate of 200 mg per kg feed in combination was found to be most effective and economic in alleviating summer stress in growing broiler rabbits.

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EFFICACY OF PROBIOTIC AND ASCORBIC ACID IN ALLEVIATING SUMMER STRESS IN GROWING BROILER RABBITS

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ABSTRACT

The research work was conducted to study the efficacy of probiotic (*Lactobacillus casei*) and ascorbic acid in alleviating summer stress in growing broiler rabbits. The study was done in the summer season from March to May. The growth performance of rabbits in summer, their physiological response to stress and the cost effectiveness of supplementing probiotic, *Lactobacillus casei* and ascorbic acid were studied.

Twenty four weaned New Zealand White rabbits were randomly selected from Rabbit unit at Krishi Vigyan Kendra, Kerala Agricultural University, Vellanikkara were utilized for the study. They were divided into four groups of six animals each. The treatments were as follows: Treatment I (T1) - Ascorbic acid (Merck) at the rate of 200 mg per kg feed was given along with the basal diet, Treatment - 2 (T2) - Probiotic, *Lactobacillus casei* (Unique Biotech) containing 10^6 colony forming units per gram of feed was given along with the basal diet, Treatment - 3(T3) - Probiotic and Ascorbic acid at the same rate as in T_2 and T_3 were given along with the basal diet and Treatment - 4 (T4) - Rabbits fed with basal diet alone.

The various climatic parameters studied were maximum and minimum temperature and relative humidity (morning and afternoon) in macro climate and maximum and minimum temperature, dry bulb temperature (morning and afternoon) and relative humidity (morning and afternoon) in micro climate. The production parameters recorded were weekly body weight, daily feed intake, average daily weight gain and feed efficiency. The physiological parameters studied were weekly respiration rate, weekly rectal temperature and monthly cortisol (faecal and serum) values (twice a month for three months). Disease incidence and mortality during the period was recorded. Cost effectiveness of supplementing probiotic and ascorbic acid was determined.

The mean monthly temperature humidity index values suggested that animals were under moderate heat stress in the first (28.45±0.16) and the second month (28.50±0.22), while no stress prevailed in the third month (26.83±0.21). By one way Analysis of variance it was found that supplementation of probiotic, *Lactobacillus casei* and ascorbic acid had a significant effect in alleviating summer stress in rabbits. The animals in the T1 group showed significantly ($P \leq 0.05$) higher overall mean daily body weight gain, overall mean feed efficiency and significantly ($P \leq 0.05$) lower overall mean respiration rate and faecal cortisol level compared to T4 animals. The rabbits in T2 treatment showed significantly higher ($P \leq 0.05$) final body weight, overall mean daily body weight gain, overall mean feed efficiency and significantly ($P \leq 0.05$) lower overall mean respiration rate compared to T4. The rabbits in the T3 group showed significantly higher ($P \leq 0.05$) final body weight, overall mean daily body weight gain, overall mean feed efficiency and significantly ($P \leq 0.05$) lower overall mean faecal cortisol level compared to T4. There was no significant difference ($P > 0.05$) between the treatments in mean feed intake, rectal temperature and serum cortisol. No disease incidence or mortality was observed during the experimental period. Supplementation of probiotic and ascorbic acid was found to be efficient in reducing the production economics. Hence it was concluded that supplementation of probiotic, *Lactobacillus casei* at the rate of 10^6 cfu per g of feed and ascorbic acid at the rate of 200 mg per kg feed in combination was found to be most effective and economic in alleviating summer stress in growing broiler rabbits.